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Award Number: DAMD17-01-1-0782

TITLE: Odors, Deployment Stress and Health: A Conditioning Analysis of Gulf War Syndrome

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REPORT DATE: September 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-09-2006	Final	1 Sep 2001 – 31 Aug 2006

4. TITLE AND SUBTITLE

Odors, Deployment Stress and Health: A Conditioning Analysis of Gulf War Syndrome

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-01-1-0782

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Pamela Dalton, Ph.D., MPH

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Monell Chemical Senses Center
Philadelphia, PA 19104-3308

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT

Troops deployed in the Persian Gulf War were exposed to an unusually diverse mix of odorous chemicals at the same time as they were exposed to physiological and psychological stressors B a scenario that research in animal models suggests will lead to the development of specific conditioned responses. The goal of this research is to investigate the extent to which people can acquire stress reactions as conditioned responses to odors and exhibit health symptoms as a result of such conditioning episodes. Thus, the paradigm investigated in this project can serve as a model system for examining and understanding the persistent symptom constellations found in GWS and other stress-mediated syndromes. Results from the first three studies strongly suggest that odor-stress conditioning can powerfully mediate elevations in hormonal status (salivary cortisol) self-reported stress, health symptoms and judged cognitive effort on memory tests, and that cognitive information about the nature of the chemical odor may enhance the stress and health symptom reports over that which is due to conditioning alone. Current studies are continuing to explore additional parameters of the odor-stress conditioning paradigm.

15. SUBJECT TERMS
odors, conditioning, stress

16. SECURITY CLASSIFICATION OF:				18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	60	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION:

The overall goal of this project was to investigate the hypothesis that the symptom constellation of Gulf War Syndrome (GWS) and other stress-mediated illnesses stemming from military deployment can be understood as conditioned responses to chemical odors encountered under stressful conditions (Bouton, Barlow & Mineka 2001). The specific goals of the overall project, which are outlined below, were to first establish that odors could be conditioned to emotional states such as stress and relaxation, then to explore specific parameters of the phenomenon, such as its persistence and whether it can be enhanced by misinformation about the hazardous nature of the odorant, and finally to investigate whether the stress-associated odor conditioning could be blocked and/or prevented.

A series of studies was conducted over a five-year period to provide answers to each of these questions. In year 1, due to delays in obtaining Army HSRRB approval to test human subjects, we focused on method development and refining of the stress and relaxation manipulations for the subsequent studies. Near the end of Year 1, having finally obtained HSRRB approval (University of Pennsylvania IRB approval had been obtained early in Year 1), Study 1 was commenced, but not completed.

The goals of Year 2 were to complete studies that were originally slated for Year 1 and to embark on and complete at least a portion of the studies originally planned to be conducted during Year 2. The delayed Year 1 studies involved investigating whether an odor could be conditioned to a psychological stressor in a conditioning session such that re-exposure to that odor alone would subsequently elicit stress and somatic responses (Study 1) and determining whether odors control responding because they serve as a discriminative stimulus for the occurrence of an adverse (or positive) outcome (Study 1A). These studies were begun at the end of Year 1 and completed in Year 2. Three additional studies were to be conducted during Year 2, investigating the effects of repeated stressor exposures and the persistence over two different retention intervals (Study 2), investigating the degree to which misinformation can sensitize responding to a stressor, post-exposure (Study 3), and exploring the degree to which an ecologically valid stressor (hazard training video) can produce heightened conditioned stress response to odors among professional emergency responders (Study 4). All three studies were begun in Year 2, but only Study 2 and study 3 were completed. Study 4 was deferred until Year

45, the no-cost extension year, due to problems with recruitment of the specified subject population (see below).

The specific goals of Year 3 were to conduct two studies to examine (1) the degree to which a conditioned response could be extinguished by re-exposure to the odor in the absence of the stressor stimulus and (2) to evaluate whether information about the toxicity of the odor could retard the extinction of the conditioned response. Those studies were completed.

The specific goals of Year 4 were to conduct two studies to examine (1) the degree to which a conditioned response could be inhibited by prior exposure to the odor in the absence of the stressor stimulus and (2) to evaluate whether the conditioned response to the odor could be blocked by pre-associating it with another odorant. Those studies were completed during Year 4. By the middle of Year 4, it was apparent that more time was needed in order to complete Study 4, so a one-year, no-cost extension was requested and granted for the purpose of completing this study. This study was completed in Year 5. The studies and their results are detailed in the sections which follow.

General Subject Recruitment and Screening

Participants were recruited using flyers, advertisements placed in local newspapers or selected from our subject database. Individuals who expressed an interest in participating in our studies were invited for an information and screening session in which they were provided with information about the nature of this study. By signing a special (independent of this study) consent form, they gave us permission to collect and store relevant demographic data in our database for the purpose of screening for eligibility for our studies. During the first session, they completed a self-report medical/occupational history and Chemical Intolerance Index (Lees, Stefaniak, Emmett, & Dalton, 2003). They were also tested for their olfactory abilities on a 7-item olfactory discrimination task, to ensure their ability to detect and process the experimental odors (Dalton, Gould, Girten, Stodieck, & Bateman, 2003). For the screening session, they received financial remuneration of $10. We relied on self-report history with confidence, because it has been our experience that our procedures pose only very minor, if any, risk of physical harm or lasting psychological distress.

Individuals (m/f) between ages 18-55, in good general health, with average olfactory

6abilities, no occupational history of chemical exposure, no chemical sensitivity, medical diagnosis of cardiovascular disease, or asthma, no pacemaker, and no psychiatric diagnoses of Chronic Fatigue Syndrome, Posttraumatic Stress Syndrome, Depression, Anxiety Disorders, Burnout Syndrome or Claustrophobia were eligible for our experiments. We regularly tested an ethnically diverse group composed of roughly equal numbers of males and females (see enrollment tables for each study). However, in order to comply with the experimental instructions, all participants had to be able to speak and understand English well.

Exclusion criteria:

Demographics: Criteria related to demographics were collected using the medical/occupational history screening form. Individuals younger than 18 years old and older than 55 years and who were non-English speakers were excluded from our studies.

Chemical Intolerance: Participants who reported regular to frequent sickness from chemical, synthetic odors (a score of 3 or above across the board, or some 4's and 5's for chemical odors) were excluded. We only had to reject two subjects based on these criteria.

Sense of smell: Participants who indicated a sense of smell much worse than most people's, or did not pass the criterion on the 7-term odor identification task were excluded from the study.

Medical criteria: Participants who answered "yes" to any of the following medical conditions were excluded from the studies: asthma, severe seasonal or perennial allergies, chronic sinusitis, deviated septum, a head injury with loss of conscience, cardiovascular (heart) disease, high blood pressure, or if they had a pacemaker.

Exposure history criteria: Individuals who indicated a prolonged (> 1 year) occupational exposure history to pesticides, industrial solvents or formaldehyde were excluded.

Psychiatric criteria: Individuals who indicated to have or have had any of the following conditions were excluded from participation: Chronic Fatigue Syndrome, Posttraumatic Stress Syndrome, Depression, Anxiety Disorders, Burnout Syndrome or Claustrophobia.

HUMAN SUBJECT PROTECTIONS

In response to initial concerns raised by the Institutional Review Board of the University of Pennsylvania and the Human Use Committee of the Army, we took additional measures to ensure the protection of subject participants in the studies described herein. In particular, one concern was expressed regarding the potential of the study manipulations introducing into participants an ongoing aversion to certain odors.

We acknowledged upfront that such a potential is present. To preclude this possibility, we employed odors that were uncommon and dissimilar to odors generally experienced in the environment. Odors that we employ are a blend of *hinoki* and *galbanum*, *leafy green* or *osmanthus*, odors, which were familiar in other cultures (Japan) but were very unfamiliar to the western world. Another odor we employed is a fragrance blend crafted for us using primarily Asian floral ingredients, which was also rated as very unfamiliar by our participants. We have had no post-experimental complaints or reports from participants alleging any persistent aftereffects from this study. No allergic responses to the fragrances were anticipated and none have been observed or reported.

No adverse events (either minor or serious) were reported to us at anytime. We had several withdrawals from the study, but these were due to conflicts with scheduling sessions, not, to our knowledge, due to any adverse reaction experienced by any subject. In order to minimize the potential for such effects, we used laboratory stressors which are rather benign (public speaking and mental arithmetic). While increased stress levels (via self-report and physiological changes) have been noted among many of the participants following the stress manipulation, such effects have appeared fairly transient. In fact, the need to minimize the impact on subjects may have to some extent compromised our ability to view the impact of odor-stress conditioning at any level close to what might be experienced in a real-world setting. Thus, the degree of the odor-stress conditioning we observed might be much greater in real-world situations. Important to our need to use deception for this study is that subjects *would* consent to some potentially stressful and unpleasant experiences in advance, without knowing exactly the nature of those experiences (which would serve to neutralize the stress value). Of course, we ensured that the subject understood that they could withdraw from the study at any time without penalty.

To ensure safety and prompt reactions in response to participant distress during the experimental procedure, 1) participants were continuously monitored through video surveillance by the investigator, and 2) heart rate and respiration frequency, which were monitored during the entire experiment, were visible to the investigator on a computer display outside of the testing chamber.

All subjects received a formal **debriefing** following the last session, in addition to their ability to ask and have answered any questions regarding the study or their reactions. The debriefing addressed the purposes of the experiment as well as issues such as the possibility of carry-over to real life. Together with the consent form, these documents provided participants with an opportunity to contact us or the Institutional Review Board in case of questions or when experiencing side-effects from participation in our study. As stated earlier, no such reports have been made.

Aim 1: Conditioning of Health Symptoms and Stress Responses to Odors

STUDY 1: The objective of this study was to investigate whether an odor could be conditioned to a psychological stressor such that a subsequent re-exposure to this odor alone could elicit the same stress responses.

Research Participants: 48 research participants (26 females, 22 males) were tested on each of two visits, each visit lasting approximately two hours in duration. Participants were assigned to the groups as listed in the design table below (n=16/gp).

Table 1. Enrollment in Study 1

	Caucasian	African/ American	Hispanic	Asian American	Other or Unknown	TOTAL
Female	**17**	**8**	**1**	**2**	**0**	**28**
Male	**12**	**6**	**2**	**0**	**0**	**20**
TOTAL	**29**	**14**	**3**	**2**	**0**	**48**

Table 2. Design of Study 1

Group	Conditioning Phase	Test Phase
1 (Congruent)	CS_b + 20 min.stressor CS_a + 20 min. relaxation	CS_a CS_b -.
2 (Incongruent)	CS_a + 20 min.stressor CS_b + 20 min. relaxation	CS_a - CS_b -.
3 (Control)	+ 20min. stressor - 20 min. relaxation	CS_a CS_b -

Design: Groups 1 and 2 were exposed to each of two odors (CS_a and CS_b) that varied in their sensory and hedonic properties. For Group 1, the odors were congruently paired with the US (unpleasant odor-stressor or neutral odor-relaxation); for Group 2, they were incongruently paired. A control condition, Group 3, was exposed to the US (stressor vs. relaxation) but without an odor, in order to evaluate the strength of conditioning that occurs to the context (room) alone.

Procedure: The study was introduced to the participant as a study about the influence of odors on cognitive performance and attention. During Session 1 (the conditioning session), research participants filled out personality questionnaires for half an hour, to allow for serum cortisol levels and any anticipatory stress related to participating in a study to decrease to a comfortable baseline level. Thereupon, the participant entered the environmental chamber, where electrodes were connected to the subject's body for 10 minutes of baseline biomonitoring of autonomic endpoints. After 10 minutes elapsed, , the subject was administered a modified version of the Trier Social Stress Task (TSST). The TSST is a mental stress provocation task consisting of a 10 minute preparation/anticipation phase and a 10 minute performance-under-stress phase (Kirschbaum, Pirke, & Hellhammer, 1993). The participant was given 10 minutes to prepare a 5-minute oral presentation which they were told would be videotaped and judged by a panel of judges. This instruction coincided with the dispersion of a detectable concentration of the Conditioning Odor, which was either hinoki/galbanum ($CS_{a)}$ or TEA ($CS_{b)}$. After 10 minutes of preparation, the experimenter announced the end of preparation and the start of the speech via intercom, and the (sham) videotape was started. Following the completion of the speech, the

participant was given a mental arithmetic task to perform for 5 additional minutes, during which the experimenter prompted the subject via intercom. After completion of the TSST/Stress-Conditioning phase, the subject was given a 10 minute rest period while the chamber odor was purged. They were then brought back to the chamber for the second half of the conditioning phase, consisting of the alternate odor paired with relaxation instructions.

Two days later, research participants returned for Session 2 (the test session). During this session, all research participants were exposed to the two odors for 30 minutes each in the chamber (in counter-balanced order) during which they completed cognitive tasks and sensory ratings while various physiological endpoints were measured.

We hypothesized that neutral or unpleasant odors that are initially paired with a stressor can elicit an increased stress response, stress-related health symptoms through conditioning mechanisms. From this, we expected that the level of autonomic arousal, perceived stress, and reported health symptoms would be significantly increased following re-exposure to the odor paired with stressor (CS+) when compared to the baseline measures in those conditions

However, we also considered that mere exposure to a stressful situation would enhance reactivity to any chemical stimulus that is subsequently experienced in that environment, in which case we might see enhancement of autonomic reactivity over baseline in Group 3 as well. This effect may be slightly larger during exposure to CS_b, due to its negative hedonic value.

Measures: The primary dependent measures were self-reported ratings of stress and associated salivary cortisol levels; heart rate, reported health symptoms and self-rated and objective performance on the California Verbal Learning Test. We collected ratings of odor intensity, irritation and annoyance to verify processing of the odor stimulus; exploratory measures included respiratory rate and startle evocability.

The following endpoints were measured *at both conditioning and test sessions*:

*Stress***:** Salivary samples for cortisol assessments were obtained upon arrival (Baseline 1: T-40), just prior to entering the chamber (Baseline 2:T-10), 10 minutes after entering chamber (Baseline 3:T0), 10 minutes into the preparation for the TSST (T+10), 10 minutes into the performance phase of the TSST (T+20), and 10, 20, and 30 minutes into the relaxation phase (T+30, T+40, and T+50). Subjective ratings of perceived stress were rated on the Labeled Magnitude Scale at

the same time-points when saliva samples were obtained. The one change we instituted in this procedure that became apparent during pilot tests was the addition of a swish and spit with mineral water immediately before each saliva sample in order to overcome the effects of 'dry mouth' and to obtain sufficient saliva for analysis.

Odor, irritation and annoyance intensity ratings: While in the chamber, the subject rated the intensity of the odor, sensory irritation and annoyance on a computer version of the Labeled Magnitude Scale every five minutes. .

Health symptoms: Health symptoms were rated on a laptop just prior to entering the chamber, and after the CS+ and CS-conditions.

The following endpoint was measured *only during the test phase*:

Cognitive Function: To evaluate the degree to which conditioned stress can impair cognitive function, we administered the California Verbal Learning Test (CVLT) as a measure of learning and memory compared with the subject's own assessment of their performance on these tests. The CVLT was obtained twice during the test session: once during each phase (stress vs. relaxing odor).

Measures of Stress Response: In this study, as well as all others conducted in this project, we have sought to obtain multiple converging measures of a clinically significant stress response, including self-reported anxiety, cortisol levels (a widely-used measure of stress response), psychophysiological parameters of arousal (including heart rate and electrodermal activity), perceived health symptoms and performance disruption on a standardized memory test (the California Verbal Learning Test). While none of these measures by themselves may be indicative of clinically significant levels of stress, we aimed to develop a profile that may be predictive of a stress response in a real-world situation. Thus, some of the measures were largely exploratory ones, while others (self-reported stress and cortisol response, memory disruption) have considerable validity in the realm of stress research.

Table 3: Timetable of events and endpoints

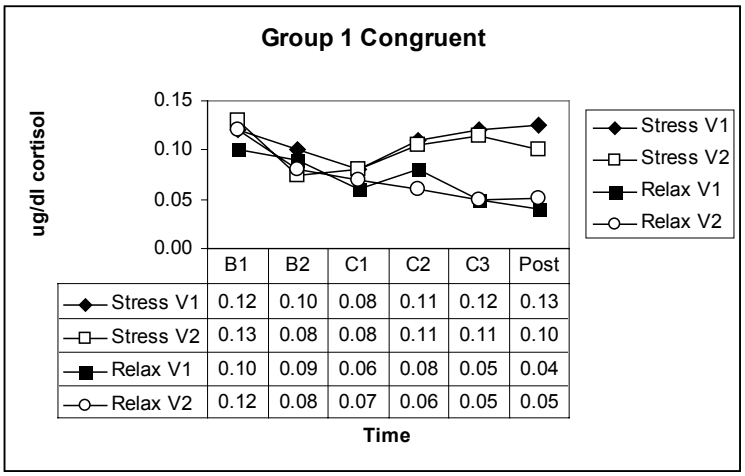
Prechamber		Chamber	Session 1: Odor a/b + Stressor Session 2: Odor a/b + Test					
	T-40	T-10	T0	T+10	T+20	T+30	T+40	T+50
Questionnaires	X							
Cortisol	Y	Y				Y		Y
VAS	Y	Y	Y	Y	Y	Y	Y	Y
Symptom		Y			Y			Y
Mood		Y			Y			Y
Intensity ratings		Y*						
HR/Resp		Y**						
Memory			Z**			Z**		

Note: The symbols X, Y, Z denote when the given measures were collected: X was measured only during Session 1, Y during both Session 1 and 2, and Z only during Session 2.
* Odor intensity ratings were collected every 5 minutes throughout the stay in the chamber
** These measures were collected continuously throughout exposure
VAS = Visual Analog Scales, HR= Heart Rate, Resp = Respiratory Rate, EDA=Electrodermal Activity,

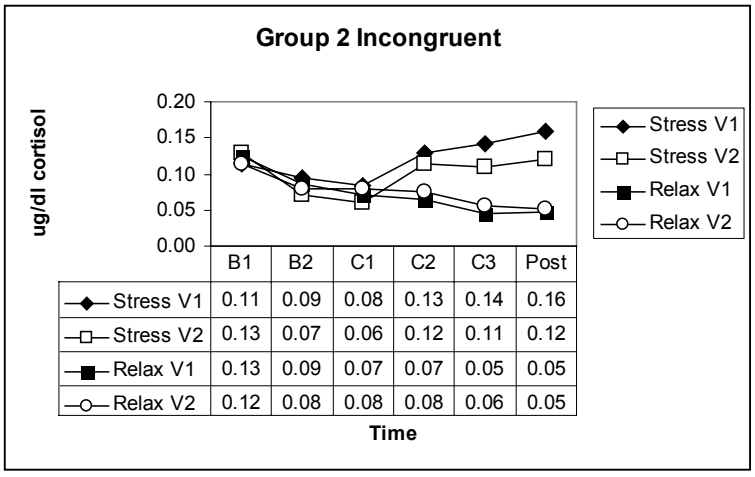
Results:

Cortisol and Self-Reported Stress: The results from the salivary cortisol analysis are presented in Figure 1 a-c for the congruent, incongruent and control conditions respectively. There was a main effect of condition: salivary cortisol levels were higher in the stressor condition across both visits than in the relaxation condition ($F_{1,47} = 5.25$, $p < .01$ and $F_{1,47} = 4.76$, $p < .01$, respectively).

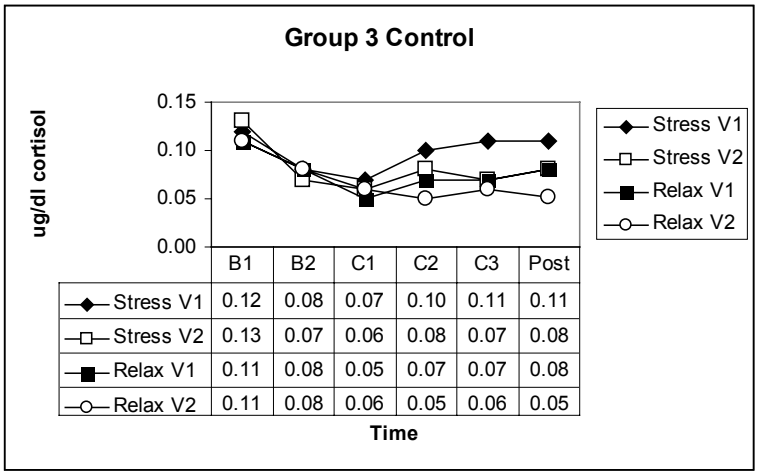
There was also a significant interaction between condition and time: Cortisol levels during and after the stressor task also reliably exceeded the second baseline (taken after the subject had spent 20-minutes filling out questionnaires); in the relaxation condition, cortisol levels were significantly lower than baseline.



	B1	B2	C1	C2	C3	Post
Stress V1	0.12	0.10	0.08	0.11	0.12	0.13
Stress V2	0.13	0.08	0.08	0.11	0.11	0.10
Relax V1	0.10	0.09	0.06	0.08	0.05	0.04
Relax V2	0.12	0.08	0.07	0.06	0.05	0.05

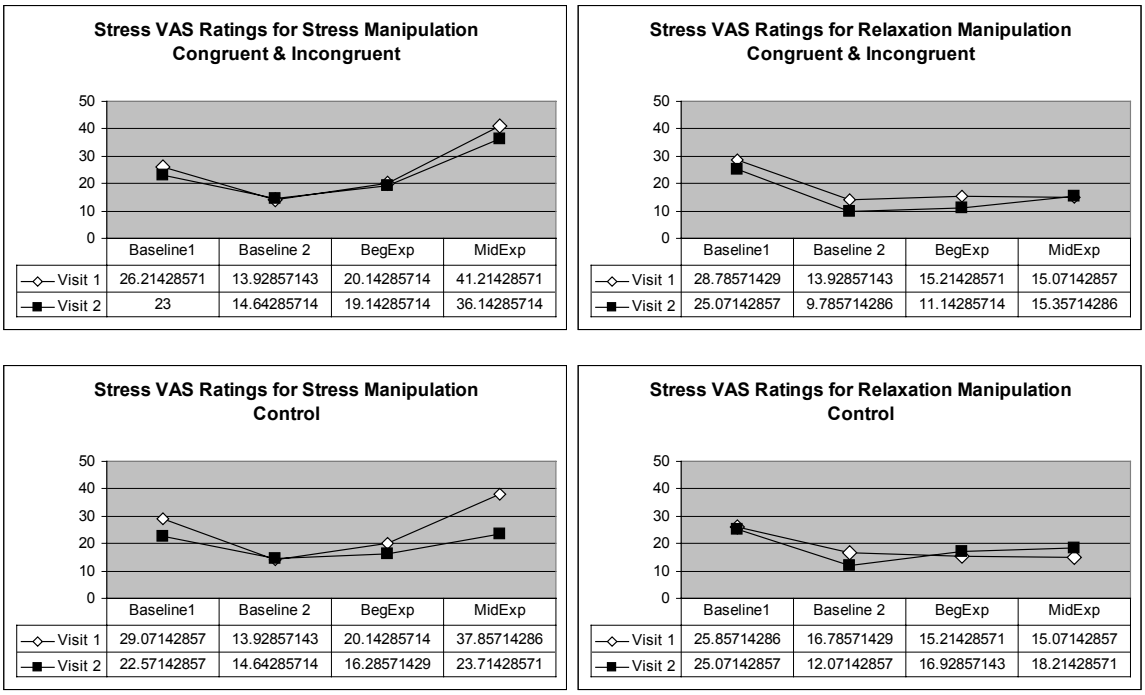


	B1	B2	C1	C2	C3	Post
Stress V1	0.11	0.09	0.08	0.13	0.14	0.16
Stress V2	0.13	0.07	0.06	0.12	0.11	0.12
Relax V1	0.13	0.09	0.07	0.07	0.05	0.05
Relax V2	0.12	0.08	0.08	0.08	0.06	0.05



	B1	B2	C1	C2	C3	Post
Stress V1	0.12	0.08	0.07	0.10	0.11	0.11
Stress V2	0.13	0.07	0.06	0.08	0.07	0.08
Relax V1	0.11	0.08	0.05	0.07	0.07	0.08
Relax V2	0.11	0.08	0.06	0.05	0.06	0.05

Figures 1 a-c Salivary cortisol levels for the three groups as a function of visit and time.



Figures 2 a-b. Self-reported stress as a function of group, visit and time.

Subjective ratings of stress: Figures 2a-2b present the subjective reports of stress at each evaluation point during Visits 1 and 2 (conditioning and test phase) for both relaxation and stress manipulations. All evaluation points were prior to the stress or relaxation manipulation, except the midpoint experiment evaluation. A preliminary ANOVA revealed no difference in the stress ratings at each time point for the subjects in the congruent and incongruent odor-pairing condition, thus for simplicity, these data are combined in Figure 2a (n=32/gp). As can be seen from the graphs, self-reported stress increased reliably during Visits 1 and 2 in the stress manipulation condition where the odor was presented at conditioning and test ($F_{3, 125} = 5.45$, $p < .01$). Absolute levels of self-reported stress during the exposure were rated as moderate to strong on the labeled magnitude scale. However, in the Control condition, stress increased reliably only during Visit 1, but not during the test phase. In contrast to the results seen with the

odor-stressor pairing, a repeated-measures ANOVA revealed that stress decreased reliably during the relaxation phase for both Visit 1 and Visit 2 ($F_{3, 61} = 4.50$, $p<.01$).

Subjective ratings of response to ODOR , Visit 2: Repeated-measures ANOVAs were performed on the subjective ratings of odor. Irritation and annoyance ratings overall were significantly higher for odors paired with the stressor condition than for odors paired with relaxation condition ($F_{1,47} = 17.89$, $p=.0001$. However, post hoc tests on the means revealed that the differences were significant only for the comparison between the conditions where odor was present during the conditioning phase; no significant differences in irritation or annoyance between stress and relaxation conditions for the control group. Across all groups, no significant differences were observed for odor intensity between the stress and relaxation conditions, thus validating the original pilot work that sought to achieve iso-intensity for the two odors.

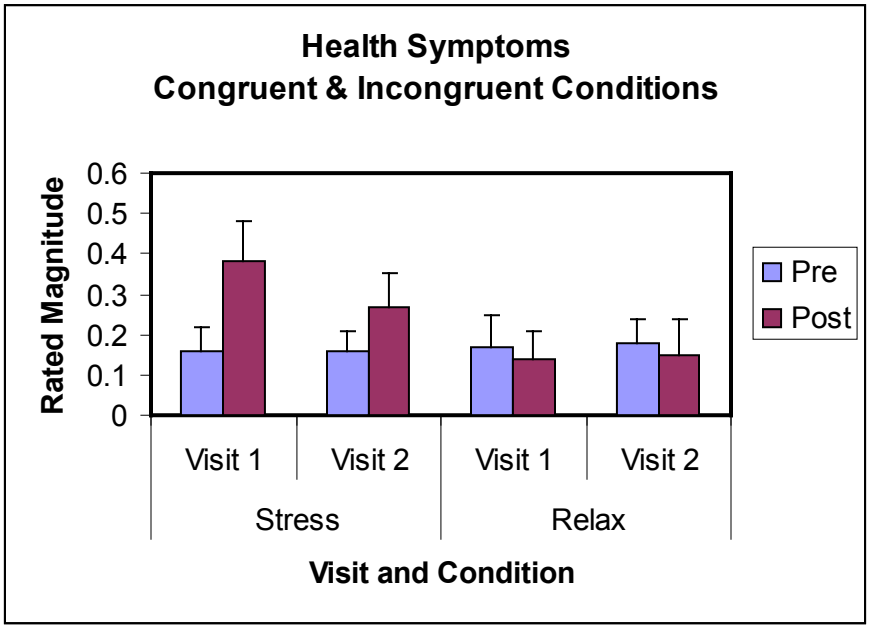


Figure 3. Rated intensity of health symptoms as a function of visit, condition and time for groups experiencing odor at both conditioning and test phase.

Health Symptoms: The rated magnitude of health symptoms before and after each exposure visit is shown in the above figure. The stress condition reliably increased the magnitude of reported health symptoms from pre- to post-exposure on both visit 1 and visit 2, whereas the relaxation condition slightly but insignificantly decreased health symptom reporting (significant interaction, $F_{(3, 18)} = 4.76$, $p <.01$.) In contrast, health symptoms in the stress condition for the

control group increased slightly, but not significantly on visit 2, thus implicating the presence of the odor at conditioning and test phase as important for the conditioned response to occur. The magnitude of increase on visit 2 was approximately 70% of the increase seen on visit 1.

Performance on CVLT: We evaluated multiple dimensions of performance on the CVLT, including number correct on free recall, number of repetitions and number of intrusions (items reported as being on the test which were not actually presented).

Free recall performance did not differ between the stress and relaxation conditions for any of the three groups, $F(1,47) = 2.11$, $p>.1$ ($M = 7.45, 7.55, 7,81$). However, the groups exhibited significantly more intrusions when re-exposed to the stress associated odor than when re-exposed to the relaxation associated odor, $F(1,47) = 7.55$; post-hoc analysis revealed this was due to differences in Group 1 & 2 who experienced odors, but not Group 3 (control) (mean difference in # of intrusions between stress/relaxation conditions = 3.4, 2,9 & .67, respectively). In addition, the groups experiencing odors on the test phase rated their memory performance as worse in the stress phase than in the relaxation phase (7.14 vs. 5.01 on a 10 point visual analog scale, respectively).

Startle evocability: We observed no difference in startle magnitude as a function of stressor or relaxation condition. Although for some individuals, the magnitude of the startle response appeared to be larger during the stress conditioning test phase, the significant variability in the magnitude of the response, (due to artifacts) across the individuals in Study 1 precluded our finding any association between startle response and stress conditioning. Because startle evocability was an exploratory measure of odor-stress conditioning and the nature of the test phase introduced so many artifacts into the physiological recordings of the eyeblink response, we have opted to exclude this measure from the remaining studies.

Study 1A - Odors as Occasion Setters for US-CS Contingency Modern studies of conditioning have shown that the results of pairing stimuli (CS-US) produce a more diverse and complex set of phenomenon than what was traditionally viewed as the conditioned response. Due to the observation of enhanced responding during test to the odors that were paired with the CS+, we can assume that the CS elicited a conditioned response (CR) because it signaled the occurrence of the unconditioned stimulus (Stress- UR). However, odors may also control responding because they "set the occasion" for the responding produced by another CS.

'Occasion setting' refers to the potential of a stimulus to clarify the predictive value of an ambiguous cue. Study 1A explored the role of conditioned odors as 'occasion setters', by replicating the basic design of Study 1 while pairing the stressor with a different CS (e.g., a 5-min video containing neutral information that was to be incorporated into the oral speech). In this paradigm, the re-presentation of the odors at test served as a signal for a positive or negative contingency between the CS and the US. In the control condition, the CS was never presented during the conditioning phase.

Table 4. Design of Study 1A

Group	Conditioning Session	Test Session
1 (Occas. Setting)	O_a CS+ Stressor O_b CS-	O_a CS_a -. O_b CS_b -
2 (control)	O_a + Stressor O_b	O_a CS_a - O_b CS_b -

Design: In this study, $Odor_a$ serves as the discriminative cue that the conditioned stimulus (CS) will be paired with an aversive unconditioned stimulus (US). In the CS+ condition, the modified Trier Social Stress Test was the US; however, the CS was a 5-min. video containing information that the subject was instructed must be incorporated into their 5-minute oral presentation. In the CS- condition, the subject was merely asked to watch the video following which they answered some multiple-choice questions about the contents. During the test session, subjects were re-exposed to the two odors (O_a & O_b) paired with the CS video. Sixteen subjects were tested in each group, yielding a total of 32 subjects.

Table 5. Enrollment in Study 1A

	Caucasian	**African/ American**	**Hispanic**	**Asian American**	**Other or Unknown**	**TOTAL**
Female	**12**	**8**	**0**	**0**	**0**	**20**
Male	**7**	**4**	**1**	**0**	**0**	**12**
TOTAL	**19**	**13**	**1**	**0**	**0**	**32**

Results: We hypothesized that the responses in Group 1 would show heightened responding to the CS+ only when it was re-presented with the odor that had been associated with the positive contingency. The results, however, were mixed. As in Study 1, salivary cortisol and self-reported stress were heightened in this condition versus the CS – condition (0.19 vs. 0.11 ug/dl for CS+ and CS-respectively), but health symptom reports did not differ among the two conditions and were not higher in the "occasion' setting condition than in the control condition (all p's > .1). Although there were no differences in memory performance on the free recall task, either in percent correct or intrusions (8.5 vs. 8.7 for % correct for CS+ and CS-, respectively), participants did self-rate their performance as being lower during the CS + phase than during the CS- test phase (7.5 vs. 6.3 on the Visual Analog Scale) , similar to the results of Study 1.

Study 2: Effect of Repeated Conditioning And Retention Interval on Odor-Stress Conditioning

The duration of deployment and the prevalence of chemical odors in the Gulf War increased the likelihood that specific odors associated with stressful situations were experienced on more than one occasion, thus strengthening the associative strength between the CS and the US. The goal of Study 2 was to investigate the degree to which conditioned autonomic responses or health symptoms were enhanced by a second exposure to the CS-US contingency. Because it is also likely that different temporal delays occurred between the initial exposure to an odor and stressor, a second goal of this experiment was to explore the persistence of conditioning effects over two different retention intervals (1 day vs. 3 days).

Table 6. Design of Study 2

Group	Conditioning Phase 1 (Day 1)	Conditioning Phase 2 (Day 3)	Test Phase (Day 4)
1	CS_a + 20 min.stressor CS_b + 20 min. relaxation	CS_a + 20 min.stressor CS_b + 20 min. relaxation	CS_a - CS_b -
2	CS_a + 20 min.stressor CS_b + 20 min. relaxation		CS_a - CS_b -

3		CS_a + 20 min.stressor CS_b + 20 min. relaxation	CS_a -. CS_b -

Design: 48 subjects were recruited and assigned to one of three groups. Group 1 received each of two neutral odors paired with a stressful and a relaxing task in the first conditioning phase (Day 1); this process was repeated on the second conditioning phase (Day 4). Group 2 and 3 only receive one conditioning trial, but the interval between the conditioning trials was either 1 or 3 days in order to evaluate the persistence of the conditioning effect from one conditioning trial.

Table 7. Enrollment in Study 2

	Caucasian	African/ American	Hispanic	Asian American	Other or Unknown	TOTAL
Female	**10**	**11**	**0**	**0**	**0**	**22**
Male	**17**	**8**	**1**	**0**	**0**	**26**
TOTAL	**26**	**19**	**1**	**0**	**0**	**49**

Procedure: The procedure for each conditioning phase and test phase replicated that used in Experiment 1. At the beginning of their second session, the participants in Group 1 were told that the previous videotape failed to be recorded and the procedure would have to be repeated. On this occasion a new topic for the oral speech was assigned, and the TSST was repeated as before.

Measures: Measures of salivary cortisol, self-reported stress, health symptoms and cognition were collected and analyzed in the same manner as in Study 1.

Hypothesis: We hypothesized that the magnitudes of the autonomic stress and health symptom responses were a function of the number of CS-US pairings and time intervening between conditioning and testing. We therefore expected that the magnitude of the conditioned stress response would be largest in the Group 1, which group received two conditioning sessions,

followed by Group 3, which received the one conditioning session only 1 day before testing, and finally, Group 2 which received the conditioning session 3 days before testing.

Results: 49 subjects were tested, but one was dropped from the analysis for failure to show up at the final session and was replaced. Analysis of salivary cortisol, stress responses and health symptoms on 48 subjects (16 in each condition) revealed a small but significant difference in the magnitude of stress conditioning on Visit 2 between Groups 1 and 3 (cortisol: .25 (+/- .06) vs. .19 (+/- .03) , respectively, $F(1,31) = 5.78$, $p<.001$). There was also no reliable difference between Group 2 and 3 for salivary cortisol (.19 (+/-.05) vs. .22 (+/-.03) however, self-reported stress was higher for group 3 than group 2 suggesting that the duration between the conditioning phase and the re-exposure is important in determining the magnitude of the stress response to the odor. We did not observe any differences in the cortisol response of Groups 1, 2 or 3 during the test phase for the relaxation session.

Health symptoms were elevated in Group 1 vs. Group 2 and 3, but no differences were observed on any measure of cognitive performance except self-rated memory effort; $F_{(2,47)} = 3.21$, $p < .05$.

Study 3: Stress, Symptoms and Odor Conditioning with Cognitively-Sensitized Odors

In the Gulf War, the ongoing threat of chemical warfare may have led many of the military personnel to become hyper-vigilant in monitoring their ambient environment for the presence of chemical odors. Previous research from our laboratory has shown that attention and anxiety about the consequences of exposure to an odor can lead to higher reported levels of odor, irritation and health symptoms. The goal of this experiment was to explore whether such information or characterization of an odor could lead to higher levels of conditioned stress response to that odor.

Aim: To investigate whether a neutral odor that has been characterized as hazardous can elicit higher levels of autonomic arousal after it has been initially experienced in the context of a stressful compared with a non-stressful task.

Design: The design for this experiment replicated that of Experiment 1, but instead of using odors that were normatively-rated as neutral and unpleasant, we employed two odors (CSn1 and CSn2) that were characterized as neutral (e.g., standard odorant used in olfactory research) or

hazardous (e.g., industrial solvent/pesticide). Sixteen subjects were tested in each group, yielding a total of 48 subjects.

Table 8. Design of Study 3

Group	Conditioning Phase	Test Phase
1 (Negative Instruction)	CS_{n1} + 20 min.stressor CS_{n2} + 20 min. relaxation	CS_{n1} - CS_{n2} -
2 (Neutral Instruction)	CS_{n1} + 20 min.stressor CS_{n2} + 20 min. relaxation	CS_{n1} -. CS_{n2}.
3 (Control)	+ 20min. stressor - 20 min. relaxation	CS_{n1} - CS_{n2} -

Table 9. Enrollment in Study 3

	Caucasian	African/ American	Hispanic	Asian American	Other or Unknown	TOTAL
Female	**11**	**9**	**5**	**2**	**0**	**27**
Male	**12**	**7**	**1**	**1**	**0**	**21**
TOTAL	**23**	**16**	**6**	**3**	**0**	**48**

Procedure: The procedure was identical to that used in Experiment 1, except that before the stressful or relaxing task in which the odor was diffused into the chamber, the subject was told (by the experimenter) that they will be exposed to either "a standard odorant that has been used and approved for olfactory research" (neutral condition) and "an industrial solvent that in long-term exposures has been reported to cause some health and cognitive problems" (negative condition).

Measures/Statistical Analysis: The same endpoints were measured/analyzed as in Study 1.

Hypothesis: We hypothesized that a neutral odor that had been initially paired with a stressor would elicit increased autonomic responses and stress-related health symptoms and memory

impairment through conditioning mechanisms, but that the magnitude of the increase in response would be larger when that odor was believed to have adverse effects on health, than when that was not believed to be the case.

Results: Analysis of the salivary cortisol, self-reported stress and health symptom responses for 45 subjects (n=15/gp; 3 sets of samples were lost due to an equipment failure) indicated that stress-conditioning was enhanced in the group receiving negative instructions over the group receiving neutral instructions or the control group; $F_{(2, 42)} = 3.15$, $p < .05$. . Salivary cortisol levels during Visit 2 were 0.21, 0.10 and 0.6 mg/dl for the negative, neutral and control group, respectively. We also observed significantly higher health symptom reports for the negative group, than the neutral or control group, both at the conditioning phase and at the test session, however further analysis revealed that the higher symptom reports were limited to the cognitive subset of symptoms and the respiratory symptoms.

Study 4: "Association of Odor (CS) To Multiple Real-World Stress Stimuli

Study 4, entitled "Association of An Odor (CS) To Multiple Real-World Stress Stimuli**"** which was originally intended to be conducted during Year 2, was delayed until Year 5, under a one-year, no-cost extension. The study investigated the feasibility of a stress induction procedure other than the Trier Social Stress Test for use in the laboratory. Law enforcement, firefighters and emergency personnel watched commercially-available emergency responder training videos, which were expected to engage their belief-system and, consequently, cause stress and arousal as a US. This stressor was presumed to have more ecological validity than the Trier Test. Study 4 originally was intended to follow the design of Study 1, but initial problems with recruitment for the time commitment involved necessitated a modification, as described below. Measures of autonomic arousal, cognitive function and self-reported stress and health symptoms were collected.

Initial responses suggested a wide variability in autonomic responses to the stressors within this cohort. We analyzed changes in autonomic response to the stressor with personality subtypes (e.g., Negative Affectivity) in order to partition out some of the variation in response, if it was due to differences in personality traits.

Modification to Study Design: A change in the protocol was proposed and implemented which greatly facilitated subject recruitment. In all prior studies, incorporating both the stressor and relaxation phase in each session caused the session to exceed 2.5 hours per day (and in some cases, it exceeded 3 hours/ session). We were able to recruit very few subjects, meeting our eligibility criteria for this study, who were able to participate for the necessary time commitment. Hence, given the importance of demonstrating a conditioned stress-response to an odor cue under more environmentally realistic conditions, we pilot tested omitting the second part of the session during which odors were paired with a relaxation induction in order to keep the session under 1.5 hours per day and allow us to collect data in a reasonable pace. This was viewed as especially important given the impact on the project timetable that occurred following the delay in obtaining HUC approval during all of Year 1. However, in order to ensure that the subject was not stressed when leaving the session, we had them participate in the relaxation induction in a separate room, but without collecting physiological data and in the absence of any odor. Consequently, this did not require any significant modification to the description of the procedure in the consent form.

The table below represented the new design (strikeouts indicate changes from the prior design.).

Table 10. Modified Design of Study 4

Group	Conditioning Phase	Test Phase
1 (Congruent)	CS_b + 20 min.stressor ~~CS_a + 20 min. relaxation~~	CS_a - HR/Resp/Startle/Cog. ~~CS_b - HR/Resp/Startle/Cog~~.
~~2 (Incongruent)~~	~~CS_a + 20 min.stressor~~ ~~CS_b + 20 min. relaxation~~	~~CS_a - HR/Resp/Startle/Cog.~~ ~~CS_b - HR/Resp/Startle/Cog.~~
~~3~~ 2 (Control)	+ 20min. stressor ~~- 20 min. relaxation~~	CS_a - HR/Resp/Startle/Cog. ~~CS_b - HR/Resp/Startle/Co~~g

Design: Group 1 was exposed to the odor in the presence of the stressor for a period of 20 minutes. Due to the elimination of the relaxation condition, there was no need to test a second group with an alternate odor. A control condition, Group 2, was exposed to the US (stressor) but without an odor, in order to evaluate the strength of conditioning that occurs to the context (room) alone. During the conditioning phase and the test phase, we monitored heart rate and

respiration rate of each participant, as measures of autonomic arousal; we also evaluated subjective symptom reports and mood. The test phase utilized these measures as well as several additional dependent measures, including a test of cognitive function (short-term and general memory performance). In both conditioning and test phases we collected salivary samples 8 times in order to measure cortisol levels. Sixteen subjects were tested in each group, yielding a total of 32 subjects. Although we attempted to recruit as many females as males, there were many more males who were available to volunteer.

Procedure: The study was introduced to the subject as a study about the influence of odors on cognitive performance and attention. The timetable and schedule of dependent measures were as indicated in Table 2. During Session 1 (the conditioning session), the subject filled out personality questionnaires for half an hour, to allow for serum cortisol levels and any anticipatory stress related to participating in a study to decrease to a comfortable baseline level. Thereupon, the subject entered the environmental chamber, where electrodes were connected to the subject's body for 10 minutes of baseline biomonitoring of autonomic endpoints. After 10 minutes elapsed, the subject was shown excerpts from various training videos which portrayed the outcome of a biological/chemical warfare attack and the actions to be taken by emergency personnel under such circumstances. Group 1 received a pairing between a novel odor (galbanum, assumed to be the CS) and the film (assumed to be the stressor US) during the Conditioning Session for a duration of 20 minutes. This was followed by the test session, 2 days later. Group 2 received two sessions: the conditioning session involved presentations of the US alone followed by presentation of the CS odors alone during the test session to examine the degree to which conditioning could occur to the context (room) alone.

Table 11: Enrollment in Study 4

	Caucasian	African/ American	Hispanic	Asian American	Other or Unknown	TOTAL
Female	**4**	**4**	**0**	**0**	**0**	**8**
Male	**15**	**8**	**1**	**0**	**0**	**24**
TOTAL	**19**	**12**	**1**	**0**	**0**	**32**

Data Analysis:

Data were analyzed using 2-way Analysis of Variance (ANOVA), with a 2 (Condition: Odor vs. Context) X 2 (Session- 1^{st} vs. 2^{nd}) design.

The ages of the subjects ranged from 27 to 51 years old. Based on data analyses from prior studies, we first examined the degree to which the experimental film excerpts elicited stress during the conditioning phase. We defined this response as an increase of 10% or more during and immediately after observing the film excerpts. However, based on these criteria only 75% of the subjects experienced stress during the conditioning phase (n=24). As these data were analyzed immediately after Session 1, we then sequentially assigned these 'responders' equally to the two conditions (odor conditioning and control). The remaining 8 subjects who did not show a stress response were also equally assigned to the two conditions. (Note: Based on these more stringent criteria, we are currently re-analyzing the results of some earlier studies to determine if segregating the subjects based on their responses during the conditioning phase will increase the degree of conditioning observed on the subsequent sessions(s)).

Changes in Heart Rate: Analysis of variance performed on the heart rate data collected during session 2 revealed a significant difference between groups; $F(1,31) = 4.57$, $p < .05$, with the group who was exposed to the odor on Day 1 exhibiting increased heart rate during the second session relative to the control group. A closer look at the subgroups within each group however, revealed that for the odor group the increase in HR was greater for the responders than the 4 non-responders (M = 14% vs. 4%, respectively). This difference in HR increase over baseline was not observed among the responders and non-responders in the control group.

Subjective ratings of stress: There was a main effect of condition on perceived stress response, $F_{1,31} = 5.35$, $p < .05$, with individuals in the group who were only exposed to the odor during the first session rating stress higher in Session 2 than did individuals who were only exposed to the odor on the second (test) session.

Performance on CVLT: Disruptions in cognitive performance, especially memory tasks, can be a useful index of stress. As in prior studies, we evaluated the degree to which conditioned stress would disrupt memory processing during the second (test) session using the California Verbal Learning Test. We evaluated multiple dimensions of performance on the CVLT, including number correct on free recall, number of repetitions and number of intrusions (items reported as being on the test which were not actually presented).

Table 12: CVLT Results on Session 2

Group	Correct Recall	Repetitions	Intrusions
Conditioning	4.75	1.1	1.67
Control	5.05	.90	1.70

ANOVA analysis revealed that overall CVLT recall performance did not differ between the two groups, $F_{(1,31)} = 1.78$, $p>.1$ (M = 5.75 and 5.05). Neither did we observe any significant differences in the number of repetitions or intrusions between groups. However, it should be noted that overall memory performance was poorer in both groups than we had observed with any other cohort using the CVLT as an index of cognitive processing. However, similar to other studies we did observe that the conditioning group rated their memory performance as somewhat, albeit not significantly, poorer than the control group. This finding is consistent with results from other studies as well as with claims among GW veterans of poorer memory performance, which are not always observed when tested using standard memory measures. In brief, this suggests that stress may interact with perceived effort such that cognitive processes are judged as more effortful and less efficient, even if the outcome on performance is the same. The increased perceived effort might have detrimental implications over time or information-processing under stressful deployment situations.

Reported Health Symptoms: Participants rated a variety of health symptoms immediately before and at the end of exposure on Days 1 and 2. 25 health symptoms were rated and for analysis, classified into 7 subgroups: Autonomic nervous system, gastrointestinal, central nervous system, cognitive, respiratory, irritation. To control for bias to respond, a number of control symptoms were also rated (i.e., leg cramps, tooth pain = sham condition).

There was a main effect of condition on health symptoms, $F_{(1,60)} = 2.51$, $p>.1$. Post hoc tests revealed that only the cognitive symptoms differed between groups, at $p <.001$. No other subgroups of symptoms differed between groups. As was true for heart rate, we further examined the subgroups of responders and non-responders in each condition. Although the unequal group sizes did not permit formal analysis, the mean ratings given to the symptom surveys did suggest that the responders rated the cognitive, autonomic and respiratory symptoms higher than did the non-responders and the ratings were higher for the group that had received conditioning to the odor in addition to the context.

AIM 2: EVALUATING THE POTENTIAL FOR EXTINCTION OF THE CONDITIONED RESPONSE

Study 5: Personality Traits & Extinction of Odor-Stress Conditioning

Aim: If the unconditioned stimulus (US) never again follows the conditioned stimulus (CS), conditioning will *extinguish*. In other words, if stress never again is induced in association with the CS, the CS will lose its ability to elicit the autonomic stress response and related health symptoms (Van den Bergh et al., 1999). However, subjects who are very susceptible to stress may have become more fearful, anxious or anticipating of the odor and its effects than subjects who are not susceptible. During the Gulf War, soldiers will have varied in the extent to which they were affected by war conditions as a function of personality traits. In Experiment 5, two groups of subjects were selected-- subjects with high scores on neuroticism and negative affectivity and subjects with low scores on these characteristics (Watson & Pennebaker, 1989). Because we pre-tested and selected participants at each end of the potential range of NA, we were more likely to see significant associations of NA and chemosensory response in small samples. Both groups received stress-odor conditioning to the CS in Session 1. An extinction session followed in which subjects were exposed to the CS (odor) but not the US (stressor), to evaluate the reduction in autonomic response and health symptoms over time in general, and also as a function of personality traits, as described below.

Table 13. Design of Study 5

Group	Conditioning Session	Extinction Session	Test Session
0 - Hi NA + Neu	CS_b + 20 min.stressor	20 min. room exposure	CS_a - HR/EDA/Cog.
0 - Lo NA + Neu	CS_a + 20 min.stressor	20 min. room exposure	CS_a - HR/EDA/Cog.
1 Hi NA & Neu	+ 20min. stressor	20 min CS_a	CS_a - HR/EDA/Cog.
1 Lo NA & Neu	+ 20min. stressor	20 min CS_a	CS_a - HR/EDA/Cog.

Subjects: Sixteen subjects were tested in each group, yielding a total of 64 subjects. The ethnic and gender breakdown are presented in Table 5. Average age of the subjects was 32.4 for the females, 29.5 for the males. As other investigators have noted, a major issue with studies of NA is that individuals with high scores on this dimension tend to be unreliable subjects and do not always complete the sessions. Hence, completion of this study took twice as long as originally planned.

PROCEDURE: Subjects participated in three sessions, tested individually. The procedure for eliciting stress during the conditioning session was identical to that described in the previous studies, with subjects exposed to a neutral odor paired with a modified version of the Trier Social Stress Task (TSST) on visit 1.

Two days later, subjects returned to the laboratory for the extinction session and two days after that, for the test session. During Session 2, Group 1 was given a 20-minute exposure to the conditioning odor in the same room as which conditioning occurred, during which no measures were taken. Subjects in Group 0 were given exposure to the room, with no conditioning odor present; thus, for this group we anticipated that extinction to the odor would not occur (although extinction to the room context may have taken place). Two days following that session, they were again exposed to the conditioning odor in the same room while the various endpoints were being measured, including self-reported stress, salivary cortisol, electrodermal response, heart rate, respiration, health symptoms and memory measures.

Table 14. Enrollment of participants in Study 5

	Caucasian	African/ American	Hispanic	Asian American	Other or Unknown	TOTAL
Female	**13**	**12**	**2**	**1**	**0**	**31**
Male	**10**	**12**	**4**	**0**	**2**	**33**
TOTAL	**23**	**24**	**6**	**1**	**2**	**64**

Hypothesis: It was expected that the autonomic stress responses and health symptom reports exhibited by subjects in Group 1 would be lower (i.e., show effects of extinction) than those exhibited by subjects in Group 0, who did not have an odor extinction session. We also hypothesized that personality features would be important mediators in the magnitude and persistence of the conditioned stress response and that Group 1 (High NA and neuroticism) would exhibit slower extinction of the autonomic stress and self-rated stress response as compared with Group 2 (Low NA and neuroticism).

Data Analysis:

Using an omnibus MANOVA, we first evaluated the impact of odor (unpleasant or neutral) on the responses to the stress and relaxation sessions on Session 1 vs. Session 3. There was no main effect of odor on any of the responses, $F_{(1,60)} = 2.18$, $p > .1$ (including rated intensity and

annoyance), suggesting that both hedonically congruent and incongruent odors were equally effective (or ineffective) at influencing the unconditioned response on Session 1 and the conditioned response on Session 3.
Next, we evaluated the main effect of conditioning (stress vs. relaxation) and found a significant overall effect of conditioning type, $F_{(1,60)} = 19.5$, $p < .05$. However, while responses on both sessions to the relaxation condition differed from those in the stress condition, there was no evidence of conditioning to relaxation (as measured by responses to the odor paired with relaxation on Session 3) on any measure except odor liking, and this is likely to represent a mere exposure effect. Thus, for simplicity and to focus on the clinical condition of interest, stress, the results reported here focus only on the responses obtained in the stress sessions, comparing the group that experienced extinction to the odor with the group that did not.

Data were analyzed using a mixed-model Analysis of Variance (ANOVA), with a 2 (Condition: extinction vs. none) X 2 (NA- high vs. low) X 2 (Session- 1st vs. 3rd) design.

Subjective ratings of stress: There was a main effect of extinction condition on perceived stress response, $F_{1, 60} = 4.35$, $p < .05$ and a main effect of personality (negative affectivity) as well, $F_{1, 60} = 3.93$, $p < .05$. Individuals who were exposed to the odor during the second session rated stress lower in Session 3 than did individuals who were not re-exposed to the odor. Individuals who were high in NA ($F_{1,60} = 5.01$, $p < .05$) rated stress higher than those low in NA. Perhaps most interestingly, there was a significant interaction between extinction condition and personality ($F_{1, 60} = 6.08$, $p < .05$. Figure 5 shows the self-rated stress during Session 3 for each of the four groups separately (Group 0 received no extinction, Group 1 received extinction trial during session 2). The loss of the conditioned response to the odor was greater for individuals who were low in NA than it was for those scoring highly on the NA dimension. Similarly, the loss of the CR to the context was greater for the low NA group than for the high NA group. These results suggest that a high NA individual who develops an odor-stress association may require more extinction sessions in order to reduce the conditioned response to the odor.

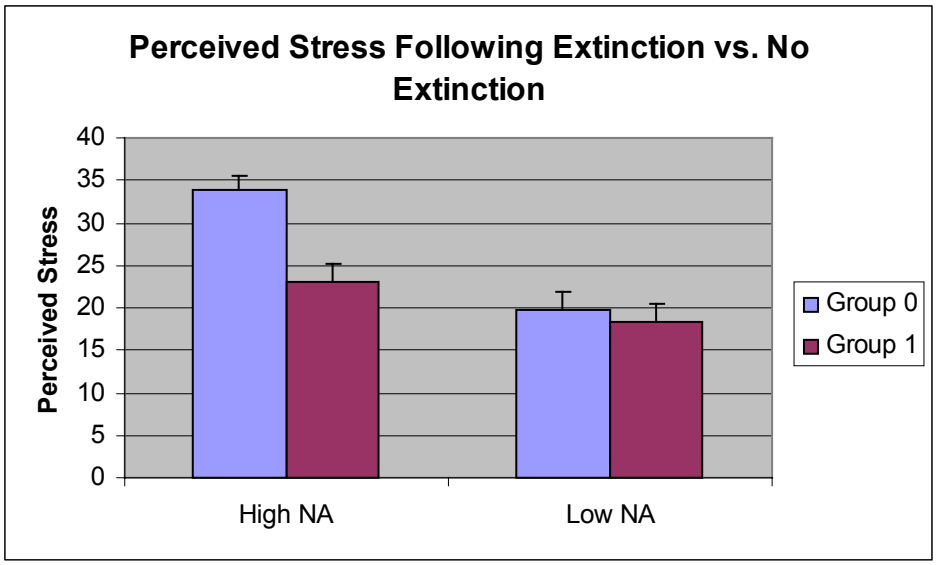


FIGURE 5: Self-reported stress on Session 3 (overall) as a function of group and personality. Stress ratings were made using a visual analogue scale ranging from 0-100 cm, where 0= no stress and 100= extreme stress.

Salivary Cortisol: Saliva samples were obtained at multiple timepoints during the first and third session in order to observe whether there were any stress-related increases in cortisol upon initial exposure and re-exposure to the odor during the test session. Due to a problem with transport of samples from storage to analytical lab, samples from only the first 48 subjects were able to be analyzed. In contrast with the self-reported stress ratings, there were no significant differences between cortisol responses as a function of extinction condition, however there was a main effect of NA on cortisol response, with individuals scoring high on the NA dimension having higher baseline cortisol and higher stress-associated cortisol on both sessions, ($F_{1,44} = 5.12$, $p < .05$). Figure 6 depicts the cortisol levels for both NA groups on Session 3. The interaction between extinction condition and NA did not reach significance, ($F_{1, 44} = 3.56$, $p = .09$). As noted in previous studies, both baseline cortisol levels and changes appear to be quite heterogenous, with some individuals showing large increases upon response to the stressor (and the subsequent re-exposure to the odor) while some individual who nonethleless, report feeling stressed do not show much of an elevation in salivary cortisol. The elevation in cortisol response for some individuals could be considered clinically significant elevations in stress response, but overall the variance we observe suggests that salivary cortisol may not be the most reliable marker of stress

across all individuals. Given the number of samples we obtain (n=8) in each session it is unlikely that we are missing any transient elevations that may be linked to the stress response, although there is still a possibility that the response is significantly delayed in some individuals and that elevations do not occur until after the experimental session has ended.

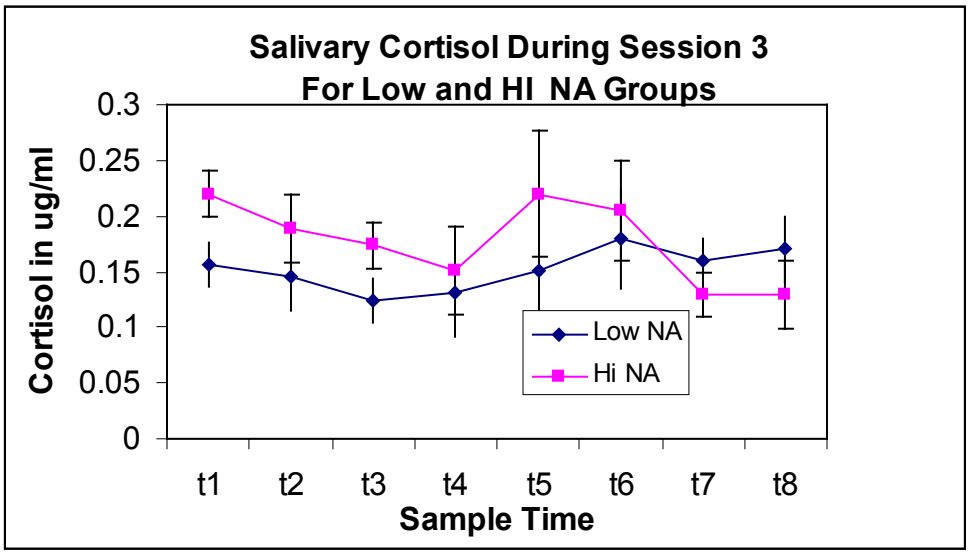


Figure 6. Cortisol response across the multiple timepoints in Session 3. Salivary samples for cortisol assessments were obtained at 10 minute intervals throughout the session: upon arrival (Baseline 1: T-40), just prior to entering the chamber (Baseline 2:T-10), 10 minutes after entering chamber (Baseline 3:T0), and at 10 minute intervals thereafter. T8 is taken 10 minutes after exposure ends.

Performance on CVLT: Disruptions in cognitive performance, especially memory tasks, is one of the primary complaints of Gulf War veterans. Accordingly, we evaluated the degree to which conditioned stress could disrupt cognitive processing using the California Verbal Learning Test. We evaluated multiple dimensions of performance on the CVLT, including number correct on free recall, number of repetitions and number of intrusions (items reported as being on the test which were not actually presented).

Table 15: CVLT Results on Session 3

Group	Correct Recall	Repetitions	Intrusions
Low NA	6.45	1.8	1.67
High NA	6.55	2.1	3.7

ANOVA analysis revealed that overall CVLT recall performance did not differ between the extinction conditions for the two groups, $F_{(1,60)} = 1.08$, $p>.1$ (M = 6.45, 6.55). However, there was a significant interaction between NA and performance type (Recall, Repetitions, Intrusions) in which the high NA group exhibited significantly more intrusions when re-exposed to the stress associated odor than did the low NA group, $F_{(1,60)} = 4.27$, $p <.05$; (mean difference in # of intrusions between NA conditions = 3.45, & 1 .77, respectively). In addition, the high NA group reported poorer memory performance on the test phase than did the low NA group (8.04 vs. 4.32 on a 10 point visual analog scale, respectively). This finding continues to be of interest given the reported claims among GW veterans of poorer memory performance, which are not always observed when tested using standard memory measures. In brief, this suggests that stress may interact with perceived effort such that cognitive processes are judged as more effortful and less efficient, even if the outcome on performance is the same. The increased perceived effort might have detrimental implications for information-processing under deployment situations.

Electrodermal response (EDR): To measure the degree of arousal orientation to the odor cue, skin conductance (EDR) was measured throughout each phase, although the analysis was confined to 1-minute epochs surrounding each of the timepoints where experimental manipulations occurred. Of greatest interest in this study was to observe whether the phasic EDR, which was time-locked to certain experimental manipulations, differed during session 3 as a function of extinction condition. Figure 7 presents the EDR response across the multiple time points of Session 3 expressed in microsiemens. Upon re-exposure to the odor in session 3, there was an increase in EDR for the group that did not receive extinction (white symbols) when compared with the group that was re-exposed to the odor without the stressor (black symbols), but this increase was not significant at the level of $p = .008$ (Bonferroni correction for multiple comparisons).

The magnitude of any phasic response to a stimulus can range from 0.5 to 5 microsiemens. Thus, while the average amplitude of the phasic response, less than 1.5 microsiemens, is not very impressive, there does appear to be a mild orienting response to the introduction of the odor in the group that had no extinction vs. the group that did. However, the amplitude of the EDR was higher at the start of the memory task, suggesting that the combination of odor + additional stress may have a greater impact than just exposure to the odor or the additional stress alone (note: no

change in EDR for the group receiving extinction at the start of the CVLT). In general, EDR response should be considered merely an exploratory measure which can signify autonomic arousal, and tying the actual levels of the phasic response to a clinically significant stress level is problematic. Hence, at the present time, we look more toward the self-report and behavioral responses to indicate the presence of conditioned and extinguished stress response.

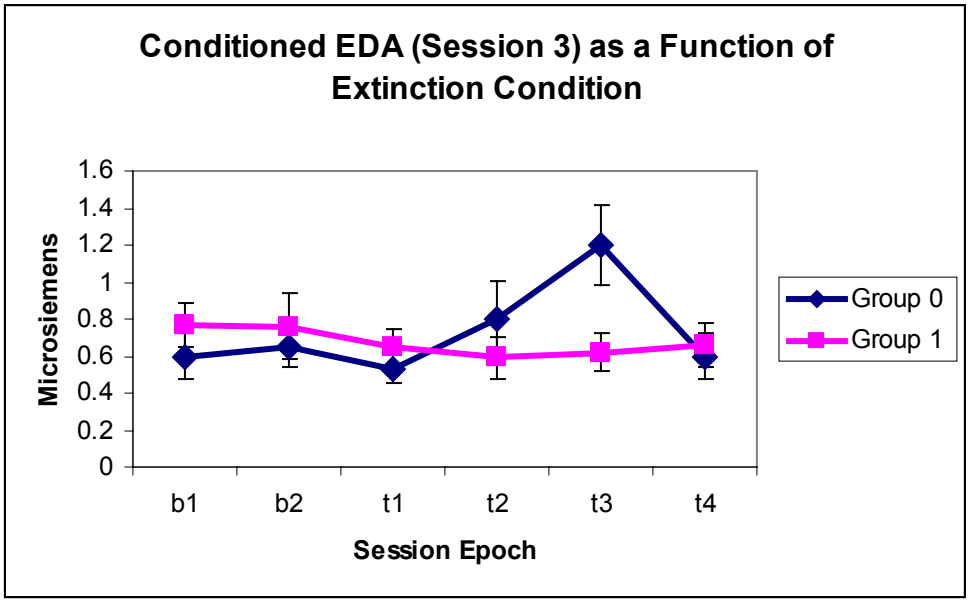


Figure 7: Electrodermal response as a function of the extinction manipulation. Filled symbols represent the averaged response of the group that did receive extinction session, Open symbols represent the no-extinction group. Typical range of EDA is 0.5-5.0 microsiemens. B1= initial baseline in chamber, B2=10 min after entry into chamber, T1=introduction of odor into chamber & buildup (respirator on), T2=removal of respirator/odor cue, T3= start of CVLT , T4=end of exposure.

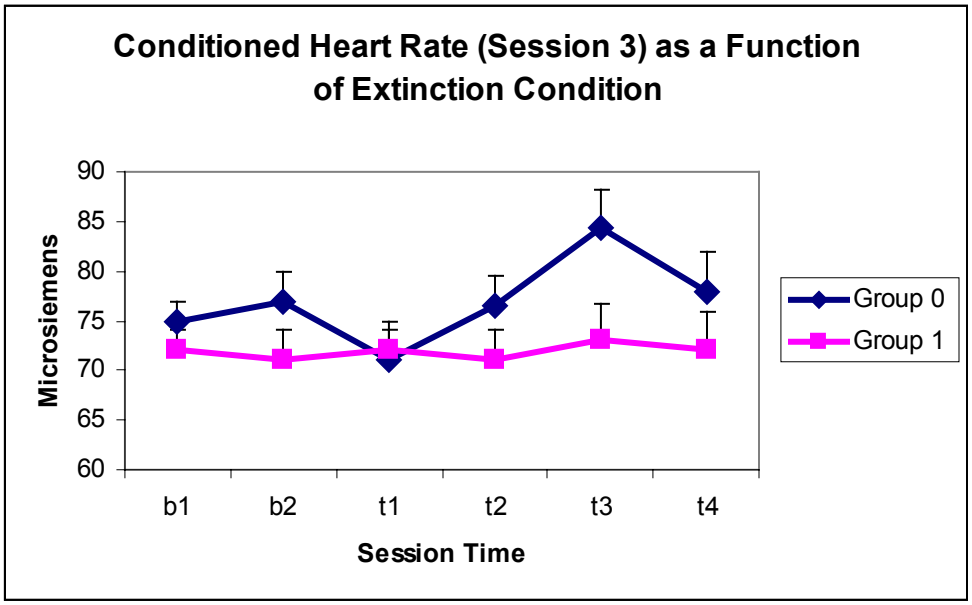


Figure 8. Heart rate during session 3 as a function of extinction condition. Time points on the X axis signify the same events as shown in Figure 2.

Heart Rate: ANOVA performed on the heart rate in session 1 and 3 revealed a marginally significant effect of extinction condition, $F_{1,60} = 5.85$, $p < .01$ ($p = .006$ after Bonferroni corrections). The average heart rates during session 3 mirrored the self-reported stress results and thus differed as a function of whether the group received the extinction session (i.e., were re-exposed to the odor) or did not (i.e., were only re-exposed to the context). Average HR (in beats per minute) for the group re-exposed to the odor during phase 2 was stable across the session and lower during re-exposure to the odor than for the group that did not receive the extinction session (M=73.8 vs. 79.8, respectively).

Reported Health Symptoms: Participants rated a variety of health symptoms immediately before and at the end of exposure on Days 1 and 3. 25 health symptoms were rated and for analysis, classified into 7 subgroups: Autonomic nervous system, gastrointestinal, central nervous system, cognitive, respiratory, irritation. To control for bias to respond, a number of control symptoms were also rated (i.e., leg cramps, tooth pain = sham condition).

There was no main effect of extinction condition on health symptoms, $F_{(1,60)} = 2.51$, $p > .1$. However, there was a main effect of personality (NA) on symptom reports, $F_{(1,60)} = 6.75$, $p < .05$)., with individuals who scored high on NA reporting significantly more health symptoms at baseline and test than individuals who scored low on NA. This is not surprising, given that NA

is closely related to health symptom perception. However, there was a significant interaction between extinction condition and health symptom groups, $F_{(6, 384)} = 3.26$, $p < .001$ (Bonferroni corrected p value of .008 for significance). Post hoc tests revealed that only the respiratory symptoms and cognitive symptoms differed between groups, both at $p < .001$. No other subgroups of symptoms differed between the group receiving the extinction session and the group that did not.

Implications of Study 5 results: Despite the fact that the overall magnitude of the conditioned response on any measure was not extremely robust in this study, we still found evidence that a single re-exposure to the odor without the stressor present was capable of somewhat reducing the conditioned response on a subsequent re-exposure. This suggests that under controlled circumstances, re-exposure to the conditioned stimulus that elicits stress may be effective in reducing the conditioned stress response. Also of interest was the observation that physiological responses to an additional stressor (memory test, CVLT) were enhanced for the group that did not receive extinction to the odor, suggesting that once an odor has come to signal a stress response, experiencing it again under stressful conditions may magnify the response. However, it should be acknowledged that the overall magnitude of the conditioned stress response was significantly lower than what might be expected under real-world deployment situations and hence, the extrapolation of this manipulation to actual situations should be cautiously evaluated. In the final studies of this project, we hope to increase the magnitude of the conditioned response in order to determine whether extinction and/or blocking of such response are a viable option for deployment stress. The major concern with such a experimental manipulation is to ensure the safety and comfort of the participants.

The observation that personality traits such as NA are related to the magnitude and persistence of the conditioned stress response to odors and environments suggests it may be a useful tool to predict individuals' reactions to combat situations. In other words, certain personality dimensions may function as mental health baselines for psychological status and suitability for given assignments. It has been suggested that, in addition to its status as a personality trait, NA can serve as a proxy for chronic stress or 'burnout'. To determine if this is the case, and to observe whether levels of chronic stress interact with phasic stress events, future studies conducted under this effort will incorporate a measure of chronic or generalized stress, as

indexed by Cohen's Perceived Stress Scale (Cohen, Kamarck, & Mermelstein, 1983) or a similar instrument.

Study 6: Extinction vs. Cognitive Sensitization

Aim: In prior studies of animal conditioning, if a CS (odor) is not re-paired with the US (stressful situation), the conditioned response to the CS odor does not always completely extinguish. Several possibilities have been suggested to account for a failure to extinguish the conditioned response. For example, extinction may be prevented from occurring if the respondent avoids remaining in the presence of the CS for a sufficiently long period of time in order to learn the lack of contingency between CS-US. Alternatively, the occurrence of the CS may elicit such a vivid memory of the original US, along with anxiety and stress responses, that the original contingency is again re-learned. And finally, additional information that may be acquired about the significance or meaning of the CS can serve as a new US, and perpetuate the original stress/autonomic response to the CS. In the latter case, exposure to media concerns about the possibility of chemical exposures in the Gulf War may have acted to sensitize individuals to the meaning and significance of the odors experienced in initially stressful circumstances.

DESIGN: To explore this possibility, we first conditioned and then attempted to extinguish the association between a neutral odor and a stressful task, using the design of Experiment 5. In this design, subjects experienced the laboratory Trier Stress Test in the presence of an unfamiliar odor (hinoki/galbanum) on the first session. On the second session, they were re-exposed to the odor without any stress manipulation. However, immediately prior to the extinction session, individuals were given one of three types of information about the odor to which they were initially exposed and would be again: Group 1 was told the odor was a natural extract (positive), Group 2 was told the odor was an agricultural pesticide additive (negative). Group 3 was not given any characterizing information (neutral). 16 subjects have been tested in each of the first two groups, 17 in the third group yielding a total of 49 subjects (data from one subject in group 3 was dropped for apparent failure to understand and follow experimental instructions). The mean age for female participants is 29.8, the mean age for males is 28.4.

Table 16. Enrollment in Study 6

	Caucasian	African/ American	Hispanic	Asian American	Other or Unknown	TOTAL
Female	**11**	**11**	**0**	**1**	**0**	**23**
Male	**8**	**18**	**0**	**0**	**0**	**26**
TOTAL	**19**	**29**	**0**	**1**	**0**	**49**

Hypothesis: We hypothesized that new, negative information about the nature and effects of the odor that an individual was exposed to on the first session may sensitize an individual and thereby retard extinction of the conditioning effects from the US-CS pairing. Specifically, we predicted that the autonomic, stress and health symptom response of subjects in Group 2, who have received the negative characterizing information about the odor, would be higher on Session 3 as compared to Groups 1 and 2. Their performance on the CVLT would also differ, as would their self-rated effort and performance.

Data Analysis:
Data were analyzed using a mixed-model Analysis of Variance (ANOVA), with a 3 (Condition: positive, negative, neutral) X 2 (Session- 1^{st} vs. 3^{rd}) design.

Subjective ratings of stress: There was a main effect of condition on perceived stress response, $F_{2, 45} = 4.17$, $p < .05$ and a marginally significant effect of session (1^{st} vs 3^{rd}, $F_{1, 45} = 2.9$, $p > .09$). This was largely due to a significant interaction between odor characterization and session ($F_{2, 45} = 5.76$, $p < .05$). Individuals who were exposed to the odor during the second session, with a negative characterization, rated stress higher than did individuals who were given a positive or neutral characterization. Figure 9 shows the self-rated stress using the visual analog scale (ranging from 0-100) during Session 3 for each of the three groups separately. The group receiving negative information about the chemical stimulus rated stress significantly higher when compared to the groups receiving positive and neutral information (M = 48, 21 and 28, respectively) on session 3, but not on session 1.

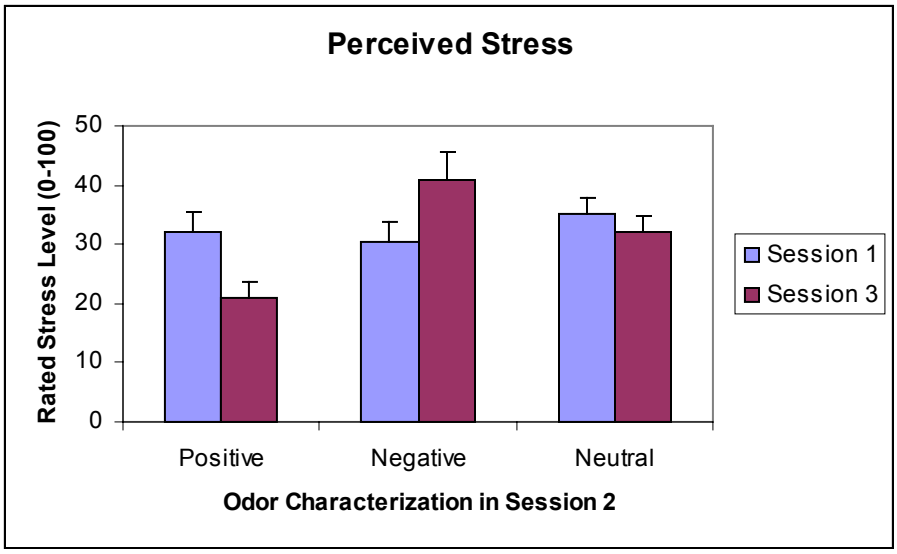


Figure 9. Rated levels of self-perceived stress in the conditioning session (1) and the test session (3) as a function of how the odorant was characterized during session 2. Ratings were made using a visual analog scale that ranged from 0 (no stress) to 100 (extreme stress).

Salivary Cortisol No differences in salivary cortisol levels were observed during session 1 among the three groups, consistent with the self-perceived stress ratings. Overall, there was a mild elevation of cortisol among the group with the negative characterization on visit 3, relative to the positive and neutral groups, the effect did not reach significance, $F(2, 45) = 1.96$, $p>.1$. Further examination of the data revealed a great deal of variability in both baseline and post-exposure cortisol levels within each group, perhaps owing to variables beyond the control of the study, such as diurnal variation, sleep schedules, etc. In fact, for some individuals in each group, their cortisol levels were highest upon arriving for Visit 3, and the values decreased over the subsequent samples. This effect has been noted in some studies prior to this and may reflect the impact of anticipatory stress related to their prior session experience.

Performance on CVLT: As in prior studies, we evaluated multiple dimensions of performance on the CVLT, including number correct on free recall, number of repetitions and number of intrusions (items reported as being on the test which were not actually presented).

Group	Correct Recall	Repetitions	Intrusions
Positive	6.75	.75	1.1
Negative	5.75	4.1	3.2
Neutral	6.25	1.1	1.9

39Analysis of variance performed on the free recall, repetitions and intrusions revealed a main effect of group (odorant characterization), $F_{(2, 45)} = 5.12$, $p < .05$. We also observed a significant interaction between odorant characterization and performance type (recall, repetition, intrusion), $F_{(4, 90)} = 2.95$, $p < .05$. The group given a positive characterization had fewer repetitions than those given the negative characterization, while the group given the negative characterization also produced the highest number of intrusions (items which were recalled, but which were not actually presented). Free recall performance during re-exposure to the odorant in session 3 did not differ as a function of how the odorant was characterized prior to the extinction session. Group Negative also reported poorer memory performance on the test phase than did the other two groups (3.42 vs. 8.01 and 6.51 on a 10 point visual analog scale, for the negative, positive and neutral group respectively), but only the comparison between the positive and negative group reached significance.

Electrodermal response: Skin conductance was again measured throughout each phase, although the analysis was always confined to averages during 1-minute epochs surrounding each of the timepoints where experimental manipulations occurred. The main comparison we have completed thus far is to observe whether the phasic skin conductance response to the reintroduction of the odorant during session 3 differed as a function of how the odorant was characterized on session 2. There is a non-significant effect of Group, $F_{(2, 45)} = 4.52$, $p = .04$ (Bonferroni corrected p value of .008), with only observable differences between the positive and negative characterizations at reintroduction to the odor, but not between the neutral and any other condition.

Heart Rate: The average heart rates during session 3 also differed as a function of whether the group received positive or negative characterization of the odorant, but the main effect of condition was only marginally significant $F_{(2,45)} = 5.93$, $p = .02$, when corrected for multiple comparisons ($p = .008$). Upon closer inspection it was obvious that this marginally significant effect was due to differences between the positive and negative group only, 75.5 bpm vs. 80.4 bpm, respectively (positive and neutral (78.2 bpm) did not differ, nor did neutral and negative).

Reported Health Symptoms: Participants rated a list of health symptoms immediately before and at the end of exposure on Days 1 and 3, using a 0-5 point scale in which 0 was used to

signify they were not experiencing that symptom while 5 indicated they were experiencing the symptom to a great degree. As before, 25 health symptoms were rated and for analysis, they were classified into 7 subgroups: autonomic nervous system (ANS) gastrointestinal (GI), central nervous system (CNS), cognitive (COG), respiratory, irritation (RI). To control for bias to respond, a number of control symptoms were also rated (i.e., leg cramps, tooth pain = sham condition).

There was both a significant main effect of odorant characterization condition on health symptoms, $F_{(2,45)} = 4.25$, $p < .05$ and an interaction between health symptom type and characterization condition, $F_{(4, 90)} = 5.48$, $p < .001$ (Bonferroni corrected p value of .007). Individuals given the negative characterization reported significantly more health symptoms in the CNS, respiratory and Cognitive category than did individuals in all other conditions. This is consistent with other studies in which we have manipulated the expectations of health effects from an odorant and suggests that even when the characterization is not explicit on the first exposure, it is possible to add to the perceived hazard level of the exposure with information available on a subsequent re-exposure.

Implications of Study 6 results: Consistent with prior studies that have shown that characterizing an odorant as potentially hazardous can exacerbate responses to that exposure on multiple dimensions (Dalton, 1996; Dalton, Wysocki, Brody, & Lawley, 1997), we found that mischaracterizing the odorant prior to the extinction phase did retard extinction of conditioned stress on some measures, for the group that had the odorant characterized as 'negative'. This suggests that belief systems, whether intrinsic or influenced by social or media cues, may be playing a role in the persistence of any adverse response to a conditioned stressor. While the study does not directly address the issue of whether beliefs about chemical exposures during the Gulf War may have increased the magnitude and persistence of any stress response veterans have experienced sine then, it is highly suggestive that such a mechanism may be playing a role.

Overall Implications: It is possible that during the Gulf War soldiers began to avoid the conditioned stimulus (e.g. a specific odor), or had repeated vivid memories of it, or received information about it through the media or otherwise. All of these may have prevented an extinction response to the CS, even if it occurred in the absence of the US. We are seeking to determine whether the physiological effects in this study establish the viability of such a mechanism.

To evaluate whether the results observed in the current studies represent clinically significant levels of stress is a difficult endeavour. To be sure, there are some consistent responses under stress conditioning and re-exposure that would seem to indicate a reliable production of a stress response to an odor cue. However, the correlation between cortisol increase (or any other psychophysiological measure)and self-perceived stress or performance disruptions on memory test are inconsistent across subjects, suggesting that even in the presence/absence of a hormone response an individual may experience stress and this stress may interfere with performance and ultimately lead to health problems. The failure to find strong correlations between objective and subjective measures of stress and performance measures is not unique to these studies (e.g.,), but suggests that other variables which are not currently being measured may contribute to one or more measures of the stress response. To this end, in future studies we will obtain measures of chronic stress, as well as evaluate factors that may contribute to the stress response in general, such as sleeplessness, exhaustion, degree of social support, etc. (Dahlgren, Akerstedt, & Kecklund, 2004; Rosal, King, Ma, & Reed, 2004).

AIM 3: PREVENTING THE ACQUISITION OF A LEARNED ASSOCIATION BETWEEN AN ODOR, STRESS AND SYMPTOMS.

If conditioned responses to previously neutral odors experienced in the context of a stressful event underlie the enhanced autonomic reactivity, stress and health symptom constellations that characterize GWS, then the literature on conditioning effects in animals and humans suggests that such associations can be prevented through mechanisms such as latent inhibition and blocking (Lubow & Moore, 1959; Kamin, 1968). Such mechanisms could be easily and usefully exploited to prepare troops prior to deployment in order to prevent the development of odor-stress associations that are likely to occur in combat or other military postings. The following experiments conducted under this aim will examine the characteristics of such preventive mechanisms.

Study 7: Latent Inhibition and Prevention of Odor-Stress Associations

Latent inhibition refers to the well-established principle of Pavlovian Conditioning (Lubow et al., 1959) whereby exposure to a CS (e.g., odor) before CS-US pairings can retard the acquisition of the conditioned response to the CS, relative to that of subjects who did not have CS pre-

exposure. Two prior exposures to a CS (odor) were given. Group 1 was given exposure to the CS odor in a non-stressful (slideshow) condition and

Table 17. Design of Study 7

Group	Exposures		Test Session
1	CS_a+20 min.stressor	CS_a + 20 min. relaxation	CS_a - HR/Resp/Cog
2	CS_b+20 min. relaxation	CS_b + 20 min.stressor	CS_a - HR/Resp/Cog.

Subjects: Twenty four subjects were tested in each group, yielding a total of 48 subjects. The ethnic and gender breakdown are presented in Table 4. Average age of the subjects was 28.8 for the females, 27.6 for the males.

PROCEDURE: Subjects participated in three sessions, tested individually. The procedure for eliciting stress during the stress- conditioning session was identical to that described in the previous studies, with subjects exposed to an odor paired with a modified version of the Trier Social Stress Task (TSST). The TSST is a mental stress provocation task consisting of a 10 minute preparation/anticipation phase and a 10 minute performance-under-stress phase (Kirschbaum et al., 1993). The subject was given 10 minutes to prepare a 5 minute oral speech which was recorded on videotape to be evaluated by a panel of judges. (Unlike in the real TSST, no evaluation actually took place). The instruction coincided with the dispersion of a detectable concentration of the Conditioning Odor. After 10 minutes of preparation, the experimenter announced the end of preparation and the start of the speech via intercom, and the videotape was started. No videos of the subject's performance were actually kept or judged and each was overwritten at the start of the next session (or erased, whichever was more feasible). Following the public speaking phase, the subject was switched to the mental arithmetic portion of the task, which required the subject to perform serial subtraction aloud. Whenever necessary, the experimenter prompted the subject via intercom to increase their speed, to begin over (when a mistake was made), etc.

The procedure for eliciting latent inhibition involved exposing the individual to the to-be-conditioned odor in a non-stressful situation. Subjects were allowed to sit and watch a slideshow for 20 minutes while the odorant was dispersed into the chamber.

Subjects were alternately assigned to Group 1 or 2 depending on the week of test. Subjects assigned to Group 1 had the non-stressful condition first, followed by the stressful condition and then the test phase. Subjects assigned to Group 2 had the stressful condition first, followed by the non-stressful condition and then the test phase. Two days following the second exposure session, all were again exposed to the conditioning odor in the same room while the various endpoints were being measured, including self-reported stress, salivary cortisol, heart rate, respiration, health symptoms and memory measures.

Table 18. Enrollment of participants in Study 7

	Caucasian	African/ American	Hispanic	Asian American	Other or Unknown	TOTAL
Female	**11**	**9**	**2**	**1**	**0**	**23**
Male	**12**	**7**	**3**	**2**	**1**	**25**
TOTAL	**23**	**16**	**5**	**3**	**1**	**48**

Hypothesis: Our hypothesis was that the conditioned-stress response to the odor would be greater for those individuals who had the stress-odor pairing on the first session than for those individuals who had the stress-odor pairing on the second session. We also hypothesized that the control groups 3 & 4 (receiving the first two sessions without any odor) would not show the same level of enhanced stress responding on Session 3 as did either Group 1 or Group 2.

Data Analysis:

Using an omnibus MANOVA, we evaluated the main effect of session order (stress first vs. relaxation first) on the magnitude of the stress response in the TSST session and found no significant overall effect of order, $F_{(1,47)} = 2.8$, $p > .1$ such that stress responses (HR, Cortisol, Self-reported stress) to the TSST manipulation was not affected by whether the TSST session occurred before or after the relaxing session. However, although initial stress responding did not look different, a MANOVA performed on the final test session showed that there was a main effect of session order, $F_{(1,,47)} = 11.6$, $p < .05$.

Subjective ratings of stress: There was a main effect of session order on perceived stress response in Session 3, $F_{1, 47} = 4.95$, $p < .05$. Individuals who were exposed to the odor paired with the relaxation session first rated stress lower in Session 3 than did individuals who were exposed to the odor during the stressful session first (M=14.2 vs. 42.5). Stress ratings were made using a visual analogue scale and scored from 0-100.

Salivary Cortisol: Saliva samples were obtained at multiple timepoints during the third session in order to observe whether there were any stress-related increases in cortisol upon re-exposure to the odor during the test session. In contrast with the self-reported stress ratings, there were no significant differences between cortisol responses as a function of session order, however, there was a trend for a significant session by gender interaction, with females showing a heightened cortisol response on session 3 if they had been exposed to the odor paired with the stressor first, ($F_{1,47} = 4.36.$, $p = .08$). As noted in previous studies, both baseline cortisol levels and changes appear to be quite heterogenous, with some individuals showing large increases upon response to the stressor (and the subsequent re-exposure to the odor) while some individuals who nonethleless, report feeling stressed do not show much of an elevation in salivary cortisol. The elevation in cortisol response for some individuals could be considered clinically significant elevations in stress response, but overall the variance we observe suggests that salivary cortisol may not be the most reliable marker of stress across all individuals.

Performance on CVLT: Disruptions in cognitive performance, especially memory tasks, have historically been a salient symptom complaint from Gulf War veterans. To evaluate the role of odor-conditioned stress in eliciting cognitive impairments, we evaluated the degree to which conditioned stress could disrupt cognitive processing using the California Verbal Learning Test. We evaluated multiple dimensions of performance on the CVLT, including number correct on free recall, number of repetitions and number of intrusions (items reported as being on the test which were not actually presented).

Table 19: CVLT Results on Session 3

Group	Correct Recall	Repetitions	Intrusions
1 (Stress 1st)	7.11	1.8	3.95
2 (Relax 1st)	6.85	2.1	2.15

ANOVA analysis revealed that overall CVLT recall performance did not differ between the session order conditions for the two groups, $F_{(1,47)} = 1.58$, $p>.1$ (M = 7.11.45, 6.85). However, as before, we saw a significant interaction between NA and performance type (Recall, Repetitions, Intrusions) in which the group which had the stressor first exhibited significantly more intrusions when re-exposed to the stress associated odor than did the group who had the relaxer first, $F_{(1,47)} = 4.51$, $p <.05$; (mean difference in # of intrusions between conditions = 3.95, & 2.15, respectively). In addition, Group 1 self- reported poorer memory performance and greater effort on the test phase than did Group 2 (6.98 vs. 3.95 on a 10 point visual analog scale, respectively). This finding continues to be of interest given the reported claims among GW veterans of poorer memory performance, which are not always observed when tested using standard memory measures. In brief, this suggests that stress may interact with perceived effort such that cognitive processes are judged as more effortful and less efficient, even if the outcome on performance is the same. The increased perceived effort might have detrimental implications for information-processing under deployment situations.

Electrodermal response (EDR): To measure the degree of arousal orientation to the odor cue, skin conductance (EDR) was measured throughout each phase, although the analysis was confined to 1-minute epochs surrounding each of the timepoints where experimental manipulations occurred. Of greatest interest in this study was to observe whether the phasic EDR, which was time-locked to certain experimental manipulations, differed during session 3 as a function of extinction condition. Figure 10 presents the EDR response across the multiple time points of Session 3 expressed in microsiemens. Upon re-exposure to the odor in session 3, there was an increase in EDR for the group that had the odor paired with stress first (circles) compared with the group that had the odor paired with relaxation first (squares), but as in previous experiments, this increase was not significant at the level of $p = .008$ (the level needed for Bonferroni correction for multiple comparisons).

The magnitude of any phasic response to a stimulus can range from 0.5 to 5 microsiemens. Thus, while the average amplitude of the phasic response, less than 1.5 microsiemens, is not very impressive, there does appear to be a mild orienting response to the introduction of the odor in the group that receive the odor paired with stress first. However, the amplitude of the EDR was higher at the start of the memory task, suggesting that the combination of odor + additional stress

may have a greater impact than just exposure to the odor or the additional stress alone (note: no change in EDR for the group who had the relaxer first at the start of the CVLT). In general, EDR response should be considered merely an exploratory measure which can signify autonomic arousal, and tying the actual levels of the phasic response to a clinically significant stress level is problematic. Hence, at the present time, we look more toward the self-report and behavioral responses to indicate the presence of conditioned and extinguished stress response.

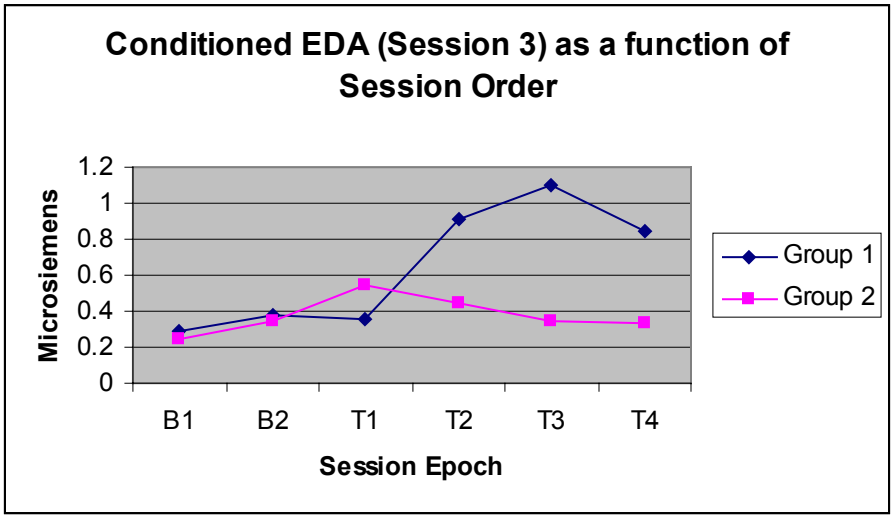


Figure 10: Electrodermal response as a function of session order. Circles represent the averaged response of the group that received the stressor first, Square symbols represent the group that received the relaxer first. Typical range of EDA is 0.5-5.0 microsiemens. B1= initial baseline in chamber, B2=10 min after entry into chamber, T1=introduction of odor into chamber & buildup (respirator on), T2=removal of respirator/odor cue, T3= start of CVLT, T4=end of exposure.

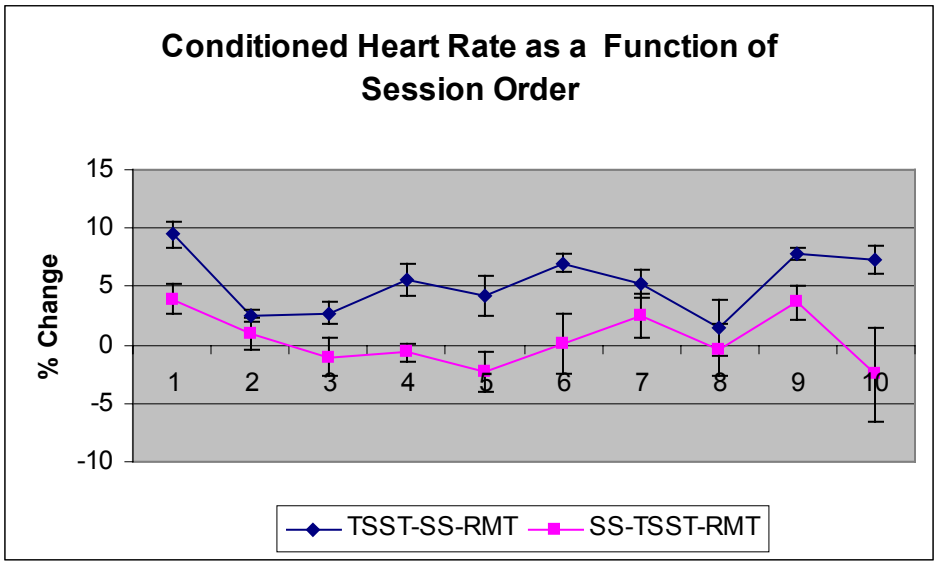


Figure 11. Heart rate during session 3 for group 1 (TSST first) and group 2 (SS first). The group experiencing the TSST paired with odor in the first session showed significantly more increased heart rate upon re-exposure to the odor on the third session.

Heart Rate: Heart rate was averaged into 10 epochs (2-min intervals during the 20-minute re-exposure to the odor in session 3). A repeated-measures ANOVA performed on the heart rate in session 3 revealed a nearly significant main effect of session order, $F_{1,47} = 15.5$, $p < 0.001$ ($p = .001$ needed after Bonferroni corrections). The average heart rates during session 3 mirrored the self-reported stress results and thus differed as a function of whether the group received the stress-odor pairing session first or second.

Reported Health Symptoms: Participants rated a variety of health symptoms immediately before and at the end of exposure on each session. 25 health symptoms were rated and for analysis, classified into 7 subgroups: Autonomic nervous system, gastrointestinal, central nervous system, cognitive, respiratory, irritation. To control for bias to respond, a number of control symptoms were also rated (i.e., leg cramps, tooth pain = sham condition).

There was a main effect of session order on health symptoms, $F_{(1,47)} = 8.21$, $p<.05$. However, a closer look at the symptom subgroups demonstrated that only several symptom clusters were responsible for the difference. There was a significant interaction between session order and health symptom groups, $F_{(6, 281)} = 5.56$, $p <.001$ (Bonferroni corrected p value of .007 for significance). Post hoc tests revealed that only the autonomic nervous system and cognitive

symptoms differed between groups, both at $p < .001$, with the group receiving the stressor session first having higher reports of symptoms on session 3. No other subgroups of symptoms differed between the groups.

Implications of Study 7 results: As was observed in earlier studies, the magnitude of the conditioned response in these studies is not large, especially when compared to what might be experienced in real-world settings. However, despite this limitation, we were able to demonstrate that the order in which an odor and stressor are experience can influence the magnitude of the conditioned response. This suggests that under controlled circumstances, prior exposure to the to-be experienced odor in a safe, non-stressful environment could possibly serve to reduce the formation of an association of that odor to stress and thus prevent the persistence of a stress-odor association in the future.

Study 8: Blocking and Prevention of Odor-Stress Associations

Study 8, entitled "Blocking and Prevention of Odor-Stress Associations", investigated the extent to which pre-associating a novel "scapegoat" odor with a stressor could neutralize the subsequent ability of other odors to become associated with stressors and prevent the acquisition of conditioned odor-stress responses. 40 subjects have been tested in three sessions in which they were exposed to either novel odors or more familiar ones, paired with stressors; the degree of odor-stress conditioning that occurred was measured by evaluation of autonomic arousal, cognitive function and self-reported stress and health symptoms.

Aim: Under many circumstances where odor-stress conditioning may be predicted to occur, it may not be possible to identify the relevant, to-be-exposed odors prior to encounter in a stressful, field or combat situation. In this case, another mechanism would be required in order to prevent the formation of an associative response to the odor. The phenomenon of *blocking* refers to the well-established finding that presenting a target CS (e.g., Odor B) in the presence of another CS (Odor A) that has been previously paired with the same US (e.g., stress) interferes with the formation of an association by the target CS. Blocking has been shown to significantly reduce the formation of chemotherapy-related food aversions among patients who were exposed to a "scapegoat" food or beverage prior to their treatment (Mattes, 1994). We predict that if a novel odor has been previously associated with a stress response or autonomic arousal, then diesel fuel,

petrochemical odor, or any odor that is subsequently experienced in the presence of that novel odor will, in general, fail to become conditioned to the US stressor and elicit the conditioned response. Thus, pre-associating an odor to a stress response prior to deployment could effectively neutralize the potential of any other odor to become associated with a stressful response. The goal of Experiment 8 was to test this assumption.

Procedure: 40 subjects were assigned to one of two groups. The novel odor (osmanthus) was identified in a pilot study from a group of candidates that have previously been rated as "very unfamiliar" by American subjects. Group 1 had one session (blocking) in which they were exposed to an odor (CS_a - osmanthus) paired with the stressor task. One day later they returned for a second (conditioning) session in which they experienced both CS_a (osmanthus) and CS_b (balsam odor) during the stressor task. On Day 3 they returned for the test of conditioning, in which they were exposed to both the novel odor and balsam odor in counter-balanced order, while the autonomic and symptom endpoints were measured (as in Experiment 1). The control group had the same experience during Sessions 2 and 3, but during Session 1 (Blocking) they were exposed to a third novel odor (CS_c – leafy green) paired with the stressor task. This odor was not experienced again. On the second session, all subjects were told mechanical failure of the videorecorder necessitated repeating the stressor task (as was done in Study 2.)

Table 20. Design of Study 8

Group	Blocking Session	Conditioning Session	Test Session
1 (Blocking)	CS_a + Stressor	CS_a, CS_b + Stressor	CS_a - HR/Resp /Cog. CS_b -HR/Resp /Cog
2 (Control)	CS_c + Stressor	CS_a, CS_b + Stressor	CS_a - HR/Resp/ Cog. CS_b -HR/Resp /Cog

Statistical Analysis: The same endpoints were measured and analyzed as in previous studies.
Hypothesis: We hypothesized that subjects in both groups would show some conditioning of autonomic stress responses and health symptoms to CS_a, but that subjects in Group 1 would show less conditioning of autonomic responses, health symptoms and cognitive impairment to balsam odor/CS_b than would subjects in Group 2. This would occur because the acquisition of

the association between balsam and stress when CS_a was present was "blocked" by the prior association between CS_a and stress.

Table21. Enrollment in Study 8

	Caucasian	African/ American	Hispanic	Asian American	Other or Unknown	TOTAL
Female	**12**	**8**	**1**	**0**	**0**	**21**
Male	**12**	**7**	**0**	**0**	**0**	**19**
TOTAL	**24**	**15**	**1**	**0**	**0**	**40**

Data from Session 3 were analyzed using Analysis of Variance (ANOVA).

Subjective ratings of stress: There was a main effect of group on perceived stress response, $F_{(1,39)} = 4.25$ $p < .05$, with subjects in the blocked group reporting less stress during session 3 than subjects in the unblocked group.

Salivary Cortisol: There were significant differences in salivary cortisol levels between the two groups on the third session, $F_{(1, 39)} = 5.75$, $p < .05$. Cortisol averaged 0.25 (+/- 0.08) ug/ml for the group that did not receive the blocking session while cortisol averaged 0.17 (+/- 0.1) ug/ml for the group that did receive the blocking session.

Performance on CVLT: As in prior studies, we evaluated multiple dimensions of performance on the CVLT, including number correct on free recall, number of repetitions and number of intrusions (items reported as being on the test which were not actually presented).

Group	Correct Recall	Repetitions	Intrusions
Blocked	8.05	.65	1.9
Unblocked	7.85	3.5	4.4

Analysis of variance performed on the free recall, repetitions and intrusions revealed a main effect of group (blocking condition), $F_{(1, 39)} = 5.36$, $p < .05$. We also observed a significant interaction between group and performance type (recall, repetition, intrusion), $F_{(4, 78)} = 3.70$, $p < .05$. The'blocked' group had fewer repetitions than the 'unblocked' group, Free recall performance during re-exposure to the odorant in session 3 did not differ as a function of blocking condition, but the number of intrusions did, with more intrusions (words generated which were not actually presented during the learning phase) in the group which did not receive 'blocking' than in the group that did. The group that did not receive the 'blocking' manipulation also reported poorer memory performance on the test phase than did the other group (4.48 vs. 6.75 on a 10 point visual analog scale, but this did not reach significance.

Electrodermal response: Skin conductance was measured throughout Session 3, although the analysis was always confined to averages during 1-minute epochs surrounding each of the timepoints where experimental manipulations occurred. The main comparison we have completed thus far is to observe whether the phasic skin conductance response to the reintroduction of the odorant during session 3 differed as a function of group. There was a non-significant effect of Group, $F_{(1,39)} = 3.82$, $p = .05$ (Bonferroni corrected p value of .008), but as reported previously, this measure is notoriously variable and thus may not confer the best indication of the strength of the conditioned effect.

Heart Rate: The percent change in heart rate from baseline to re-exposure during session 3 did, however differ as a function of group, with Group 1, having lower average heart rate than Group 2. However, the main effect of group was only marginally significant $F_{(1,39)} = 7.01$, $p = .01$, when corrected for multiple comparisons ($p = .008$).

Reported Health Symptoms: Participants rated a list of health symptoms immediately before and at the end of exposure on all days, using a 0-5 point scale in which 0 was used to signify they were not experiencing that symptom while 5 indicated they were experiencing the symptom to a great degree. As before, 25 health symptoms were rated and for analysis, they were classified into 7 subgroups: autonomic nervous system (ANS) gastrointestinal (GI), central nervous system (CNS), cognitive (COG), respiratory, irritation (RI). To control for bias to respond, a number of control symptoms were also rated (i.e., leg cramps, tooth pain = sham condition). Analysis of symptom reports on Session 3 showed that there was an overall effect of group, $F_{(1,39)} = 8.15$, $p < .05$, with the 'blocked' group reporting fewer and less intense symptoms overall

than the ‘unblocked’ group. Closer inspection revealed that the significant effect was due to symptom reports in only three subgroups: ANS, CNS and COG. Control symptoms did not differ between the two groups.

Implications of Study 8: If the relevant odors to which soldiers might be exposed during deployment cannot be identified in advance, a training scenario in which a "scapegoat" odor (one not likely to be encountered during normal living conditions) is experienced during stressful training, could be utilized. This ‘scapegoat’ odor could be used during actual deployment to "block" the formation of additional odor-stress responses.

KEY RESEARCH ACCOMPLISHMENTS

- Verified ability to induce odor-stress conditioning with a single exposure to odor under conditions of moderate laboratory stress.
- Found reliable evidence of stress-odor conditioning as manifested on increased salivary cortisol levels, self-reported stress, heightened health symptom reports (e.g., CNS, upper airway irritation) that were approximately 80% of the magnitude of increase observed during the initial conditioning phase (US-stressor).
- Consistent with reports of Gulf War veterans, self-rated memory performance was judged to be poorer for the test during which the stress odor was presented than for the test during which the relaxation odor was presented, although objective memory performance did not differ.
- In addition, although free recall performance on the tests of memory did not differ as a function of conditioning phase (stress vs. relaxation), the number of intrusions (i.e. items that were not presented at study but were produced during test) was higher during the stress test phase than the relaxation phase. Also consistent with reports of Gulf War veterans, reported effort during the memory test was higher when tested with the stress-associated odor than the relax-associated odor, even though objective performance did not differ between these two conditions.
- Observed that a single presentation of the conditioned stimulus (odor) in the absence of the stressor was sufficient to reduce the magnitude of the stress response on a subsequent re-exposure. However, the amplitude of the conditioned response was not very robust, and thus extrapolations to real-world conditioned stress responses must be made cautiously.
- Found reliable differences in the extent to which a conditioned stress response was manifest as a function of personality traits. Individuals high in NA showed less extinction of the conditioned response than did individuals low in NA.

- Observed that pairing the odor with a relaxing experience prior to associating it with a stressful experience significantly reduced the ability of the odorant itself to elicit a stress response on subsequent re-exposure. This outcome has relevance for preventing odor-stress associations for individuals who might be deployed in novel odiferous settings where stress and other adverse experiences are likely.
- Observed that if a stressor is pre-conditioned to an odor that is not again encountered, this can reduce the possibility that another novel odor can be associated with the stress response.

REPORTABLE OUTCOMES:

2 presentations were made at the Association of Chemoreception Sciences Meeting in Sarasota, Florida in April 2004 reporting the results of Study 2 and 3.

1 presentation was made at the Association of Chemoreception Sciences Meeting in Sarasota, Florida in April 2005 reporting the results of Study 6.

A presentation was made at an NSF sponsored conference in Arlington, VA in August, 2005, reporting the results of Studies 6 and 7.

A presentation was made at the Association of Chemoreception Sciences Meeting in Sarasota, Florida in April 2005 reporting the results of Study 7 and 8.

Several manuscripts are in preparation summarizing the results of all studies.

Abstracts

C. Maute, M. Gould & P. Dalton (2004) ODOR CONDITIONING AND THE STRESS RESPONSE, Chemical Senses, 24, 145.

P. Dalton, C. Maute, F. Naqvi (2004) ODOR PERCEPTION AND JUDGED PROBABILITIES OF HEALTH RISK, Chemical Senses, 24, 154.

P. Dalton, C. Maute (2005) Odor conditioning and resistance to extinction. Chemical Senses, 25, 158.

P. Dalton, C. Maute, L. Sitvarin (2006) Blocking the effects of odor-stress conditioning Chemical Senses, 26, 131.

Overall Conclusions

The goals for studies conducted in Aim 1 were to determine the ability of odors that were paired with a stressful situation to subsequently elicit a negative response that was greater than that which occurred only to the experimental context. Results from these studies suggested that odor-stress conditioning does readily occur and is a more robust form of conditioning than is odor-relaxation conditioning. This response can be seen in self-reported annoyance to the odor, self-reported stress ratings during odor exposure, and judged, but not objective, performance on a cognitive learning and memory task. Hormone levels, such as cortisol, exhibited a more variable relationship to the experimental manipulations and any of the other endpoints. This outcome may be due to the multiple sources of influence over hormone levels and suggest that salivary cortisol may not be the most robust way of indexing mild-to-moderate stressors in the laboratory.

The goals of studies conducted in Aim 2 were intended to evaluate additional parameters of the odor-stress conditioning response, specifically, the degree to which such response might be enhanced by repeated exposure to the stress-odor combination and the persistence of the stress response over different intervals following the initial odor-stress pairing. Under this aim, we also sought to investigate the degree to which more ecologically valid stimuli (emergency worker training video excerpts) could induce a stress response to an odor that was simultaneously experienced. Results from these studies suggested that many parameters of the odor-stress conditioned response interacted with personality factors such as negative affectivity (NA), such that individuals high in NA appeared to require a greater number of exposures to the odor in order to extinguish the conditioned response. The study conducted to mimic real-world stressors yielded somewhat mixed results in that we determined that only 75% of the individuals tested exhibited a stress response to the experimental manipulation; hence, our results are weaker than would have been optimal and any conclusions we could draw from this study are limited in their generalizability to real-world situations.

The goals of studies conducted in Aim 3 were to evaluate in the laboratory, the ability to prevent the development of odor-stress conditioned responses. The results of the studies thus far, although marginal, appear to hold some promise for identifying possible strategies that might be used inhibit the formation of an odor-stress association prior to deployment.

To evaluate whether the results observed in the current studies represent clinically significant reductions in the levels of stress is a difficult endeavor. To be sure, there are some

consistent responses under stress conditioning and re-exposure that would seem to indicate a reliable production of a stress response to an odor cue. However, as we have observed in the past, the correlation between cortisol increase (or any other psychophysiological measure) and self-perceived stress or performance disruptions on memory test are inconsistent across subjects, suggesting that even in the presence/absence of a hormone response an individual may experience stress and this stress may interfere with performance and ultimately lead to health problems. The failure to find strong correlations between objective and subjective measures of stress and performance measures is not unique to our studies (e.g., (Karkow et al., 2004), but suggests that other variables which are not currently being measured may contribute to one or more measures of the stress response.

Although the research was conducted on non-military personnel in laboratory situations, the findings do appear to have relevance for understanding the often diffuse symptom complex that individuals report following their exposure to situations where stressors are initially experienced in the presence of novel odors (e.g., many different types of deployment situations). Thus, the protocol used in this project can serve as a useful laboratory-based model system for examining and understanding the persistent symptom constellations found in GWS and other stress-mediated syndromes.

The Gulf War exemplified a trend of increasing threat posed by chemical warfare and biological weapons, accompanied by improved access through media, internet and other resources, to information about the nature and hazard potential of these agents. The combination of actual threat of exposure to dangerous agents and their odors, and the knowledge about the hazard potential and health effects of exposures, introduces a new factor to modern warfare that needs to be acknowledged and understood. This factor is the increased likelihood of a syndrome of health symptoms brought on by potential exposure to probably hazardous odors, their feared effects, and their stress potential. There is a continuing prospect for GWS –like illness, triggered by odors, among deployed military personnel and other individuals exposed to similarly stressful situations. The studies conducted under this research project may serve to shed light on useful mechanisms for alleviating and preventing such outcomes.

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								Personnel List											
	GRANT 83055-01																		
	DR. DALTON																		
Employee Last Name		**Employee First Name**	**GL Code**	**11/30/01**		**12/31/01**	**01/31/02**	**02/28/02**	**03/31/02**	**04/30/02**	**05/31/02**	**06/30/02**	**07/31/02**	**08/31/02**	**09/30/02**	**10/31/02**			
Dalton		Pamela	5001	15.00%		15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%				
Maute		Christopher	5050	100.00%		100.00%	100.00%	100.00%	100.00%				100.00%	100.00%	100.00%				
Smeets		Monique	5003	75.00%		75.00%	75.00%												
Opiekun		Richard	5003						100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%				
	GRANT 83055-02																		
	DR. DALTON																		
Employee Last Name		**Employee First Name**	**GL Code**	**10/31/02**		**11/30/02**	**12/31/02**	**01/31/03**	**02/28/03**	**03/31/03**	**04/30/03**	**05/31/03**	**06/30/03**	**07/31/03**	**08/31/03**	**09/30/03**			
Dalton		Pamela	5001	15.00%		15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%			
Maute		Christopher	5050	100.00%		100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%			
Opiekun		Richard	5003	100.00%		100.00%	100.00%	100.00%	20.00%	20.00%	20.00%	20.00%	20.00%	20.00%	20.00%	20.00%			
Gould		Michelle	5010					25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%			
Diamond		Jeanmarie	5050									100.00%	100.00%	100.00%	100.00%	100.00%			
Fetter		Jessica	5052										100.00%	100.00%					
	GRANT 83055-03																		
	DR. DALTON																		
Employee Last Name		**Employee First Name**	**GL Code**	**10/31/03**		**11/30/03**	**12/31/03**	**01/31/04**	**02/28/04**	**03/31/04**	**04/30/04**	**05/31/04**	**06/30/04**	**07/31/04**	**08/31/04**	**09/30/04**	**10/31/04**		
Dalton		Pamela	5001	15.00%		15.00%	15.00%	40.00%	40.00%	40.00%	40.00%	40.00%	40.00%	15.00%	15.00%	15.00%	15.00%		
Maute		Christopher	5050	100.00%		100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	20.00%	20.00%		
Opiekun		Richard	5003	20.00%		20.00%	20.00%	60.00%	60.00%	60.00%	60.00%	60.00%	60.00%	60.00%	60.00%	60.00%	60.00%		
Gould		Michelle	5010	25.00%		25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%		
Diamond		Jeanmarie	5050	100.00%		100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%				
Naqvi		Ferheen	5050						50.00%	50.00%	50.00%	50.00%	50.00%	50.00%	50.00%	50.00%			
Vannice		Kirsten	5052							100.00%	100.00%	100.00%	100.00%	100.00%	100.00%				
	GRANT 83055-04																		
	DR. DALTON																		
Employee Last Name		**Employee First Name**	**GL Code**	**11/30/04**		**12/31/04**	**01/31/05**	**02/28/05**	**03/31/05**	**04/30/05**	**05/31/05**	**06/30/05**	**07/31/05**	**08/31/05**	**09/30/05**	**10/31/05**			
Dalton		Pamela	5001	15.00%		15.00%	15.00%	15.00%	15.00%	15.00%	15.00%				15.00%	15.00%			
Maute		Christopher	5050	20.00%		20.00%	20.00%	20.00%	20.00%	20.00%	20.00%				25.00%	25.00%			
Gould		Michelle	5010	25.00%		25.00%	25.00%	25.00%	25.00%	25.00%	25.00%								
Schultz		Christopher	5052			50.00%		50.00%	50.00%	50.00%	50.00%								
Sitvarin		Laura	5050						50.00%		50.00%	50.00%	50.00%	50.00%	50.00%	50.00%			
Petrova		Maja	5003											20.00%		20.00%			
					11/30/05	**12/31/05**	**01/31/06**	**02/28/06**	**03/31/06**	**04/30/06**	**05/31/06**	**06/30/06**	**07/31/06**	**08/31/06**	**09/30/06**	**10/31/06**			
Dalton		Pamela	5001	15.00%		15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%	15.00%					
Maute		Christopher	5050	25.00%		25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	25.00%	100.00%					
Gould		Michelle	5010																
Schultz		Christopher	5052																
Sitvarin		Laura	5050	50.00%		50.00%	50.00%	50.00%	50.00%	50.00%	50.00%	50.00%	50.00%	100.00%					
Petrova		Maja	5003	20.00%		20.00%	20.00%	20.00%	20.00%	20.00%	20.00%	20.00%							
Weis		James	5052								100.00%		100.00%						
Jacquot		Laurence	5005										20.00%						